MAR 2 2 1988

379-B-12

D 15.13:86-241

DNA-TR-86-241

PHYSIOLOGICAL MECHANISMS OF ACUTE INTESTINAL RADIATION DEATH

K. L. Jackson
J. P. Geraci
University of Washington
Radiological Sciences SB-75
Department of Environmental Health
Seattle, WA 98195

1 June 1986

COMPLETED

Technical Report

CONTRACT No. DNA 001-83-C-0009

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REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION: UNCLASSIFIED

1b. RESTRICTIVE MARKINGS:

2a. SECURITY CLASSIFICATION AUTHORITY: N/A since Unclassified

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE: N/A since Unclassified

3. DISTRIBUTION / AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S):

5. MONITORING ORGANIZATION REPORT NUMBER(S): DNA-TR-86-241

6a. NAME OF PERFORMING ORGANIZATION: University of Washington

6b. OFFICE SYMBOL (If applicable):

6c. ADDRESS (City, State, and ZIP Code):
Radiological Sciences SB-75
Department of Environmental Health
Seattle, WA 98195

7a. NAME OF MONITORING ORGANIZATION:
Director
Defense Nuclear Agency

7b. ADDRESS (City, State, and ZIP Code): Washington, DC 20305-1000

8a. NAME OF FUNDING / SPONSORING ORGANIZATION:

8b. OFFICE SYMBOL (If applicable): STBE/Harrison

8c. ADDRESS (City, State, and ZIP Code):

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DNA 001-83-C-0009

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
62715H	U99QMXM	K	DH006393

11. TITLE (Include Security Classification): PHYSIOLOGICAL MECHANISMS OF ACUTE INTESTINAL RADIATION DEATH.

12. PERSONAL AUTHOR(S): Jackson, Kenneth L. and Geraci, Joseph P.

13a. TYPE OF REPORT: Technical

13b. TIME COVERED: FROM 830601 TO 860601

14. DATE OF REPORT (Year, Month, Day): 860601

15. PAGE COUNT: 114

16. SUPPLEMENTARY NOTATION: This work was sponsored by the Defense Nuclear Agency under RDT&E RMSS Code B350085466 U99QMXMK00040 H2590D.

17. COSATI CODES

FIELD	GROUP	SUB-GROUP
6	18	
18	3	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number):
Gastrointestinal Syndrome
Gamma Ray Exposure
Radiation Death
Neutron Exposure

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The overall objective was to clarify the role of fluid and electrolyte loss, bile-duct ligation, radiation-damaged intestinal mucosa, bacterial toxemia and their interrelationships on radiation-induced gastrointestinal death. Using specific pathogen-free CD-1 male rats, this study found that endogenous enteric bacteria did not play a significant role in pure intestinal radiation death. Bile acids, per se, were shown to play little role in intestinal radiation death but that the inability of the denuded mucosa to absorb fluid and electrolytes, thereby producing hypovolemic shock was the major mechanism.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION: UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL: Sandra E. Young

22b. TELEPHONE (Include Area Code): (202) 325-7042

22c. OFFICE SYMBOL: DNA/CSTI

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

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SECTION 1

INTRODUCTION

Until recently acute intestinal death has not been a principal concern of large dose, whole-body human exposures to gamma radiation, or mixtures of gamma and neutron radiations, because acute hematopoietic death occurs at doses lower than those producing intestinal death. Hematopoietic injury becomes of major clinical concern at gamma doses greater than about 250 rad and intestinal injury at gamma doses of the order of 1,000 rad (1,2), particularly when high dose rates are involved (3). However, in the case of exposure to a relatively pure beam of fission and 14 MeV neutrons from an enhanced-radiation weapon (4,5), intestinal injury will play a much greater role with respect to survival time, type of medical treatment required, and probability of survival. This greater importance of intestinal injury with neutron irradiation has been well documented (6-8).

In our laboratory, previous studies with mice have shown that the RBE of fast neutrons (8 MeV average energy) relative to 250 kVp x-rays for acute intestinal death is 2.4 compared to an RBE of 1.1 for acute hematopoietic death (9). This suggests that survival of an individual exposed to 500 rad of neutrons will require not only treatment of hematopoietic injury but also successful treatment of otherwise lethal intestinal damage. In this hypothetical example the biological equivalent dose (rad dose x RBE) is 550 rad for the hematopoietic system and 1,200 rad for the intestinal syndrome. In this example, successful treatment of the intestinal syndrome is essential since intestinal death precedes in time hematopoietic death. Furthermore, if the average neutron energy is lower than 8 MeV, lethal intestinal injury may occur without lethal hematopoietic injury. Evidence for this is the rapid increase in the neutron RBE for the LD50/4-5 day as neutron energy decreases, i.e., RBE = 1.4 for 15 MeV neutrons (10), RBE = 2.4 for 8 MeV average energy neutrons (9), RBE = 2.3 for 6 MeV average energy neutrons (11), RBE = 4.3 for 1.2 MeV mean energy neutrons (12). This is to be contrasted with a quite low RBE for hematopoietic injury over this range of neutron energy, i.e., RBE = 1.1 to 1.2 for 15 MeV neutrons (13,14), RBE = 1.1 for 8 MeV average energy neutrons (9), RBE = 1.8 and 1.0 for fission neutrons (15,16).

Although there is considerable information concerning the acute hematopoietic radiation syndrome in humans, involving both marrow transplantation (17) and conventional medical treatment (18,19), this is not so for the intestinal radiation syndrome. Excluding the recent accident at Chernobyl, USSR, only four, or possibly five, human cases of acute intestinal radiation death have been studied, each with lethal outcomes prior to the expected time of death from hematopoietic injury (20-23). (At Chernobyl as many as 10 people may have died from acute GI injury.) Complicating this is the fact that after more than 20 years of animal experimentation there still is considerable disagreement concerning the primary physiological mechanism(s) leading to acute intestinal radiation death. There is no disagreement that radiation damage to the intestinal epithelium leads to lethal physiological derangements. It is the nature of these physiological derangements that is in question and is of importance. If this were properly understood, efficient treatment could more readily be initiated which would prolong survival to the extent needed for repair of the intestine by means of cellular repopulation of the mucosal epithelium. There is reason to believe this would occur if survival time can be prolonged sufficiently since the intestinal mucosa has been shown to be capable of recovery in rodents after doses as high as 5,000 rad of gamma radiation (2,24). It is expected that similar cellular repopulation will occur after neutron irradiation (25). This concept is supported by unpublished data obtained in our laboratory which show that the rate of cell repopulation in the mouse small intestine, as measured by change in DNA content, is similar after whole-body exposures to 350 rad of neutrons and 900 rad of x-rays, doses which produce approximately equal intestine damage. In addition, we have observed mucosal regeneration in the locally irradiated small intestine after a neutron dose of 1,000 rad.

The physiological mechanisms which have been most frequently proposed as the primary ones leading to intestinal radiation death are fluid and electrolyte loss (26-29), action of bile salts on the radiation damaged intestinal mucosa (30-32), alterations in the vascular system (33-36), and bacterial toxemia (37,38).

Whether or not loss of fluid and electrolyte via the intestinal tract is a primary mechanism still is in question. We have earlier reported that following supralethal whole-body radiation exposures there is increased sodium and water loss which is sufficient to cause death (27). We concluded this results from failure of the intestine to reabsorb the water and sodium in bile (28,39) due to loss of net sodium transport from intestine to blood, and eventually to massive leakage of sodium and water from blood to intestinal lumen (40). This in turn has been reported to cause decreases in the exchangeable sodium space, extracellular fluid space, and plasma volume (41,42), changes which may be sufficient to cause shock and death. In contrast, other investigators have reported there is no, or insignificant, loss of body sodium due to supralethal irradiation (43,44), and relatively little decrease in total plasma volume (44) or vascular space (35). They conclude that loss of fluid and electrolytes in the form of diarrhea is not the primary physiological mechanism of death. Further support for their conclusion was failure to show that electrolyte replacement therapy was effective in preventing intestinal death (44).

Following our original finding (28) that bile duct ligation delays acute intestinal radiation death, other investigators proposed that an important physiological mechanism involves the action of bile salts on the irradiated mucosa, which in turn leads to severe diarrhea. Evidence for this concept is that supralethally irradiated rats which had their bile diverted to the exterior did not develop diarrhea three to four days after exposure, even if saline in a volume equal to that secreted in bile was introduced into the intestine (30). Introduction of bile salts, however, did result in diarrhea in bile duct cannulated animals. It also was shown that bile salts have a mucous depleting action on the intestinal epithelium and this may alter mucosal transport of water and electrolyte since it leads to diarrhea (31). Further work by these other investigators has led to the suggestion that this action of bile salts in the supralethally irradiated rat is the result of an increase in intestinal lumen bile salt concentration due to defective intestinal absorption (32). In contrast to this proposed role of bile salts, we subsequently reported data which show that after supralethal irradiation net leakage of sodium across the rat intestine from blood to lumen occurred in the absence of bile, and bile salts had no significant effect on the magnitude of the leakage (39). We did, however, confirm Sullivan's finding (30) because, when sodium solution was injected into the intestine, bile acids increased the flow of sodium from blood to intestinal lumen. From this it is clear that the role of bile in the intestinal syndrome has not been satisfactorily resolved and more detailed studies on the action of intestinal bile acids after irradiation have been needed.

Although several studies have shown there are alterations in blood flow after supralethal irradiation (33,34,36), no clear evidence is available to indicate this plays an initiating physiological role in acute intestinal death. Rather, what may be more important and which has received little attention is the role of the bacterial flora of the intestinal tract. Evidence that this may be important is the finding that treatment of supralethally irradiated rats with electrolyte solution is ineffective unless combined with antibiotics. Taketa (37) has shown that injection of salt solution together with penicillin and streptomycin carried 1,000 R, abdomen irradiated rats well beyond the time of acute intestinal death and this did not occur when only salt solution was injected. Also, antibiotics given alone were not very effective. Similar results in dogs have been reported (45). Dogs which were whole-body gamma irradiated with doses up to 1,600 R were carried well beyond the time of acute intestinal death by infusions of electrolyte solutions, antibiotics, plasma, protein hydrolysates and vitamins. In this regard, it is of interest that endotoxin has been detected in the livers of mice between 24 and 72 hours after 850 rad x-irradiation (46). This endotoxin may be released from the intestinal tract and, if in sufficient quantity, could lead to deleterious effects (47). Thus, the role of the intestinal microbial flora in the acute intestinal radiation syndrome also needs to be examined in much greater detail.

It should be pointed out that studies of germfree mice do not clarify the role enteric bacteria may play in increasing the severity of diarrhea after irradiation. Supralethally irradiated germ free mice exhibit a lethal intestinal radiation syndrome with diarrhea present but both death and diarrhea occur at a later time than in conventional mice and these responses require a larger radiation dose (2,48). The delay in the times of diarrhea and death in germ free mice is associated with a slower rate of cell renewal in the intestinal epithelium (2,49) which is believed to be the result of lack of conversion of taurocholic acid to cholic acid in the intestinal lumen (50,51). In conventional mice enteric bacteria produce cholic acid from the taurocholic acid secreted in bile and cholic acid results in a shorter life span of the epithelial cells of the villi. The question that needs answering, however, is whether enteric bacteria cause effects in the irradiated, conventional animal which contribute to acute intestinal radiation death. In this respect it is important to recognize that as early as 13 hours after supralethal abdominal irradiation of rats there is no net transport of water or sodium by the gut (40), presumably due to radiation injury in the mature, non-cycling epithelial cells of the villi, which results in loss of active transport of sodium. Thus, ordinarily small effects of bacterial endotoxins or bile acids on intestinal transport might become major effects with respect to net leakage of fluid and electrolyte in the irradiated, conventional animal. An action by endotoxins on net intestinal leakage of fluid and electrolyte in irradiated rats is suggested by the report that E coli endotoxin causes an increase in capillary permeability to fluid and to macromolecules having molecular weights as large as 250,000 (52). In the irradiated animal with intestinal injury there is net leakage from blood to intestinal lumen not only of fluid and electrolyte but also of macromolecules (53). Further, since changes in bile acid levels can affect the growth of microorganisms (54), and since bile or bile acids cause depletion of mucus from the irradiated intestinal epithelium (31) which normally retains the bacterial flora (55), there may be complex interactions between bile or bile acids and the enteric flora in the development of the intestinal radiation syndrome.

This brief discussion is an attempt to point out that: (a) successful clinical management of the intestinal radiation syndrome recently has become of greater importance and (b) some of the information needed to accomplish this is lacking. Under the present contract we have conducted a series of experiments to study the complex set of physiological changes that culminate in acute intestinal death resulting from neutron or gamma irradiation. It is believed this work has added to our knowledge concerning in vivo tissue responses to radiation injury and will aid in the development of more effective treatment of this syndrome in humans.

SECTION 2

METHODS AND MATERIALS

Animals. Specific pathogen-free (SPF) CD-1 male rats (140-160 g) were shipped in boxes equipped with sterile filters from Charles River Laboratory (Portage, MI). Upon arrival, animals were checked for Pseudomonas by culturing homogenized fresh fecal samples on Cetrimide Selective Agar (56). The animals were housed in the University of Washington vivarium in a humidity and temperature controlled room with a 12-hr light-dark cycle. Prior to receiving the animals, the entire room was fogged with an insecticide and bactericide and sealed for several hours. Animals were housed four to a cage with a filter-top cover. All cages, bedding, and food were autoclaved. Autoclaved distilled water, acidified (pH 3) with HCl, was used as drinking water. To further reduce the possibility of contamination, each animal rack was isolated in a ceiling to floor hood. All personnel entering the room or handling cages, water bottles, food, or animals wore sterile disposable shoe covers, surgical masks, and gloves. Spot serological, microbiological, endoparasitic, and ectoparasitic checks on selected animals by the University of Washington Division of Animal Medicine indicate no new pathogenic organisms infected the colony during the course of the experiments. At the Armed Forces Radiobiology Research Institute (AFRRI), animals were housed in microisolation cages (Lab Products).

Decontamination of the GI tract. A variation of the technique described by van Bekkum et al. (57) was used to decontaminate the GI tract. Nine days before irradiation, drinking water was withheld from the animals for 2 days to increase their thirst and ensure consumption of water containing antibiotics. At the University of Washington these animals were transferred to wall to ceiling isolation hoods separate from those housing the conventional rats. One week prior to irradiation, these animals received autoclaved acidified distilled water containing nonabsorbable antibiotics (neomycin 4 g/liter, bacitracin 2.7 g/liter, streptomycin 4 g/liter) as drinking water. To make the water more acceptable to the rats, glucose (50 g/liter) was added to the water. After irradiation, the antibiotic concentration in the drinking water was reduced by one-half and the glucose was eliminated. Conventional rats received the same treatment and water without antibiotics. This procedure resulted in 96% of the animals being decontaminated as measured by the number of the animals having sterile feces, as shown by culturing feces in brain heart infusion enrichment broth. The few animals that were not decontaminated (<5%) refused to drink the antibiotic containing water, became severely emaciated, and were eliminated from the experiment. Only completely decontaminated animals were used.

Contamination of the intestinal tract with Pseudomonas Aeruginosa. Pseudomonas organisms were obtained from Dr. Richard I. Walker of the National Naval Medical Center and cultured overnight on Cetrimide Selective Agar plates. The bacteria were swabbed off the plates with sterile Q-tips and suspended in phosphate-buffered saline (PBS) solution at the desired concentration. Bacteria were administered to the rats by oral intubation of 1 ml of suspension 2 days before irradiation.

Irradiation and dosimetery. Four unanesthetized rats were restrained in well-ventilated disinfected Plexiglas tubes in front of a 10-cm-thick lucite backscatter block. Sterile gloves were used in handling animals. The animals were continuously rotated (25 rpm) around their longitudinal axes during both neutron and gamma ray exposures. Photon irradiations were done with a 1600-Ci 137-Cs Picker therapy unit (mean dose rate 41 rad/min, 37.5 cm source to midanimal distance). The maximum variation in the photon dose between any two animal positions was less than 5% of the mean value. Neutrons of approximately 8-MeV average energy were produced at the University of Washington cyclotron by 22 MeV deuteron bombardment of a beryllium (Be) target. During neutron irradiation the midline of the animals was 152.5 cm from the Be target. At this position the dose rate was 20 to 30 rad/min. The gamma contribution to the dose was less than 5% as measured with a magnesium-argon chamber. Neutron doses reported are total doses and include the gamma ray component. The maximum variation in the neutron dose between any two animals' positions was less than 10% of the mean. Both neutron and gamma ray doses were measured with a calibrated 1 cc A-150 tissue equivalent ionization chamber placed in animal holders filled with tissue equivalent fluid with the same geometry used in the animal irradiations (see Appendix II for more detail).

At AFRRI, photon irradiations were done using a 60-Co source. Animals restrained in well-ventilated Plexiglas boxes were bilaterally exposed 390 cm from the 60-Co source at a dose rate of 0.43 Gy/min. Fission neutron irradiations were done with the AFRRI TRIGA reactor (58) operating at 5 kW in the steady-state mode. During fission neutron exposure 10 rats were constrained in cylindrical Plexiglas (3.2 mm thick) tubes. The animals were continuously rotated (1 rpm) around their longitudinal axes during exposure. To minimize irradiation from room scatter, the entire irradiation setup was surrounded by a 5 cm thick lead cave. Attenuation of fission gamma rays and fission product gamma rays was achieved by positioning a 15 cm lead shield between the reactor tank wall and the open side of the cave. The neutron and gamma ray spectra have been calculated and measured by Verbiniski et al. (59,60). The mean neutron and gamma ray energies in the cave were 0.68 and 1.8 MeV, respectively (61). Radiation doses were measured at the center of Lucite rat phantoms using Exradin 0.5 cc tissue-equivalent (TE) and magnesium ion chambers operating with methane base TE gas and argon, respectively. The dose rate at the midline was 0.4 Gy/min with 90% of the dose due to neutrons and the rest gamma rays. The midline gamma and neutron dose rates were, respectively, 1.76 and 0.72 times the free-in-air and neutron kerma rates. These changes are attributable to attenuation of neutrons and the production of gamma rays by thermal neutron capture by hydrogen in the phantom and holders. Neutron doses reported are total midline doses and include the gamma ray component. All animal irradiations with fission neutrons were monitored with ionization chambers and sulfur activation foils.

Intestinal weight and DNA content. Decrease in small intestine weight and DNA content after irradiation was used as an index of mucosal breakdown (62). For this purpose, at various times after irradiation, animals were sacrificed, the abdomen was surgically opened, and the small intestine was removed, slit longitudinally, rinsed in cold isotonic saline, blotted dry, weighed, frozen in liquid nitrogen and stored in a deep freezer at -10°C.

Subsequently, the samples were thawed, homogenized and DNA analyses performed on aliquots of the homogenate by a diphenylamine colorimetric procedure (63) after extraction of nucleic acids with hot trichloroacetic acid solution (64).

Diarrhea. The onset of diarrhea was determined by the presence of wet amber-colored fur around the anus.

Microbiological assays. Blood and liver samples taken for microbiological assay were removed from animals under aseptic conditions. Blood sepsis was determined by culture of 2 ml of blood obtained by cardiac puncture in 20 ml of peptone broth in blood culture tubes (Becton-Dickinson) for 7 days. For determination of liver sepsis and endotoxin, liver samples were homogenized in pyrogen-free water (2/1:w/v) and centrifuged at 1000g for 15 min. One milliliter of supernate was added to peptone broth tubes and these were incubated for 7 days for sepsis determination. Endotoxin was assayed by mixing 0.1 ml of supernate with an equal volume of Limulus Amebocyte Lysate (LAL) (Assoc. Cape Cod, Inc., Woodshole, MA) and incubated at 37°C for 1 hr (65). Endotoxin in the liver was also assayed by injecting the supernatant into actinomycin-D (ACT-D)-sensitized mice (66). Positive cultures for sepsis were sent to the University of Washington Division of Animal Medicine Diagnostic Laboratory for identification of the organism(s) present. Total bacterial concentration in homogenized feces, liver, spleen, small intestine, cecum, or intestinal tract contents was determined by culturing serially, tenfold diluted (with PBS) samples on BHI agar plates. Pseudomonas concentrations were determined by culturing similar samples on Cetrimide Selective Agar plates.

Surgical procedures and postoperative care. All surgical implantations of cannulae were performed while the animals were under light ether anesthesia. Cannulae and sutures were gas sterilized with ethylene oxide for 12 to 15 hours. Prior to all surgical incisions the surgical area was shaved and swabbed with Betadine solution. Animals with external cannulae were housed one per cage.

Cannulation of the duodenum and peritoneal cavity and injection of fluid and electrolytes, taurocholic acid, antibiotics, and cholestyramine. Cannulae were inserted into the lumen of the duodenums of 24 hour fasted rats, 5 to 7 days prior to irradiation to allow sufficient time for the surgical wound in the intestinal tract to heal. A 1 to 2 cm incision was made in the upper right quadrant of the abdomen in each animal, the stomach located, and retracted. A small incision was made in the stomach wall with scissors and a cannula made from silastic medical grade tubing (0.03 inches ID by 0.065 inches OD) was threaded through the stomach incision, through the pyloric sphincter and into the duodenum. The stomach incision was closed and the cannula anchored to the stomach wall with a "00" silk suture. The free end of the cannula was then routed through the abdominal wall and underneath the skin to the intrascapular region and brought to the surface through a puncture made by a 13 gauge needle. The incision was closed using wound clips (Clay Adams). A shortened (0.25 inch) 18 gauge IV Teflon catheter needle (Becton-Dickinson) was inserted into the free end of the cannula and secured to the animal's neck by means of a binder clip. The same procedure was used to insert a cannula into the peritoneal cavity one day after irradiation through a 1 cm midline incision in the abdomen.

Starting one day after irradiation different amounts (5 or 10 cc) of lactate Ringer solution (LR) (Travenol Laboratories Inc.) were injected into the small intestine or peritoneal cavity via these cannulae 3 times a day at 8 hour intervals by temporarily connecting a 5 cc syringe to the teflon needle on the back of the animals. In some animals, starting 3 days after irradiation, the LR solution was supplemented with antibiotics (Neomycin Sulfate, 1g/l; Bacitracin, 1g/l; Streptomycin Sulfate, 1g/l; Gentamicin Sulfate, 1g/l; Penicillin-G, 1g/l; Metronidazole, 1g/l). Fluid and electrolyte therapy was continued until death of the animal.

The administration of cholestyramine was done by duodenal injection of 3.5 to 5 ml of LR containing either 0, 0.1 or 0.2 grams/ml of resin at 8 hour intervals starting immediately after irradiation. The amount of resin injected per day was 5 to 15 times that necessary to bind 95% of bile acids secreted into the intestine by a normal rat in one day (unpublished data). Cholestyramine treatment was continued until death of the animal.

Bile duct ligation (BDL). A 1 to 2 cm midline abdominal incision was made in each animal, and the bile duct exposed by retracting the intestine and lifting the frontal lobes of the liver. Ligation of the bile duct was accomplished with a single silk ligature placed about 5 mm distal to the point at which the hepatic ducts from the liver converge to form the common bile duct. The ligature was placed between the most distal bifurcation of the bile duct and the point at which pancreatic secretion enters the bile duct. The incision was closed with wound clips. All bile duct ligations were done one day after irradiation.

Choledochostomy. One day after irradiation the bile duct was surgically exposed as described above. Cannulation of the bile duct was accomplished by inserting PE-10 polyethylene tubing into the common bile duct distal to the bifurcation and securing it with a silk ligature. The free end was routed underneath the skin, exteriorized in the intrascapular region, and run down the back and secured to the skin with wound clips in a position that allowed complete drainage of the bile to the outside of the animal.

Red blood cell volume (RBCV), plasma volume (PLV), hematocrit (HCT), extracellular fluid space (ECS), total body water measurements, and plasma diamine oxidase activity (DAO). Measurement of circulating red cell volume and total plasma volume was done according to the methods recommended for measurement of these parameters by the International Committee for Standardization in Hematology (67), with modifications for use in rats. Total vascular volume was taken as the sum of the red cell and plasma volumes. Rat erythrocytes were labeled with 51-Cr as sodium chromate using the citrate wash method. Commercially available 125-I human albumin will be used to measure plasma volume. With the rat under ether anesthesia, a known amount of activity of each of these tracers was intravenously injected via the external jugular vein. Three minutes after injection blood was withdrawn by cardiac puncture. 51-Cr and 125-I in plasma aliquots was measured with a sodium iodide crystal gamma spectrometer.

To measure the hematocrit, aliquots of Li heparinized blood in capillary tubes were centrifuged at 3,000g for 30 minutes. Plasma DAO activity was measured by the enzymatic formation of pyrroline from 3-H putrescine as described by Ely et al. (68). In the male rat the mucosa is the primary source of DAO activity in plasma, hence plasma DAO can be used as an index of the breakdown and recovery of the intestinal mucosa following irradiation.

The total body ECS was measured using 51-Cr EDTA as an extracellular fluid space marker (69). Tritiated water was used to measure total body water. For these measurements rats were surgically opened under anesthesia and the two renal hila ligated to prevent the kidney removal of 51-Cr EDTA. The abdomen was closed with surgical clips and 51-Cr EDTA and tritiated water in lactate Ringer's solution (0.2 ml) injected into the jugular vein. After two hours a cardiac blood sample was taken, centrifuged to separate the plasma, and 20 ul of plasma mixed with scintillation fluid and analyzed for tritium activity in a Beckman liquid scintillation counter. Another 20 ul of plasma was analyzed for 51-Cr activity in a Packard gamma spectrometer.

Statistics. Animals were continuously checked for diarrhea and survival at four hour intervals starting immediately after irradiation for the first 5 days and then at eight hour intervals. The Litchfield Wilcoxon (70) probit method was used to calculate the 50% lethal dose and 95% confidence interval. Median values for survival time and for the time of onset of diarrhea are reported in this study. For those instances in which animals died prior to developing diarrhea, median values were obtained using the life table method of Peto et al. (72). Binomial probabilities were used to determine 95% or greater confidence interval for median survival time or median time for onset of diarrhea (72). The Wilcoxon Rank Sum test (72) or the Log Rank test (71) were used to test for differences among experimental groups with respect to survival or time of onset of diarrhea. A 5% significance level was accepted as the criterion of statistical significance.

SECTION 3

RESULTS

Preliminary studies to evaluate methods for antibiotic decontamination of GI tract (Experiment 1 of Work Statement).

Pilot studies indicated that rats will not drink water containing high doses of antibiotics (neomycin, streptomycin and bacitracin) and the antimycotic agent, pimaricin. Subsequent studies involving different antibiotic solutions with and without pimaricin indicate that this drug was the major cause of the animals' refusal to drink the water. (A personal communication with Dr. Van Der Waaij of the University of Groningen, The Netherlands subsequently indicated that, unlike mice, yeast infections are not a problem in rats and therefore the pimaricin should be omitted). We also found that the addition of glucose at 5% concentration to the antibiotic drinking water significantly improved the acceptability of water. Further improvement in the consumption of antibiotic containing water was noted when the animals were made thirsty by withholding water for 2 days, which was used as a standard procedure in this phase of the study.

Administration of neomycin, streptomycin, and bacitracin in the drinking water for one week successfully decontaminated the GI tracts of rats as shown in Figures 1 and 2. Figure 1 shows the decrease in the number of bacteria per fecal pellet as a function of antibiotic treatment time. By the 6th day there was less than 1 bacterium detected per fecal pellet (usually zero), which was taken to indicate sterile feces. In Figure 2 is shown the percent of animals that excreted sterile fecal pellets as a function of time after antibiotic treatment. 100% of the animals were successfully decontaminated by the 7th day using this technique.

Damage to intestinal mucosa after gamma and neutron irradiation and correlation with levels of bacteria present (Experiment 2 of Work Statement).

Radiation-induced breakdown of the mucosa, as measured by decrease in intestinal weight and DNA content as a function of time after various doses of neutrons and gamma rays, is shown in

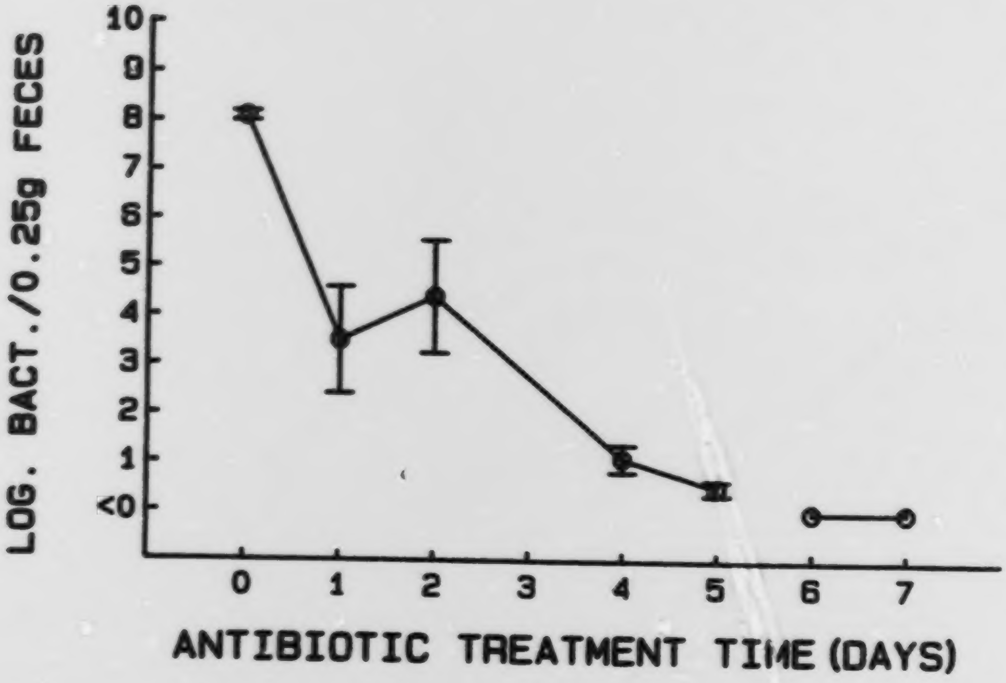

Figure 1. Fecal bacteria concentration as a function of time after beginning antibiotic decontamination of the GI tract. Each point represents the log mean bacterial concentration of 10 rats. Error bars are standard errors. Omission of bar indicates that the standard error was smaller than the point symbol.

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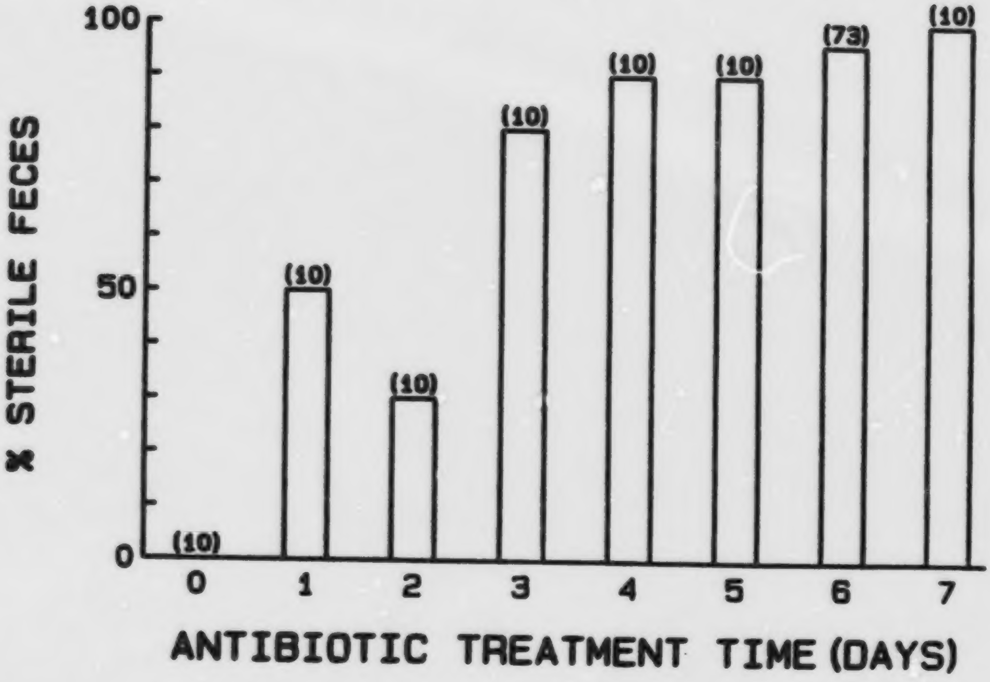

Figure 2. Percentage of animals with sterile feces at different times after beginning antibiotic decontamination of the GI tract. The value in parenthesis at the top of each bar indicates the number of animals tested.

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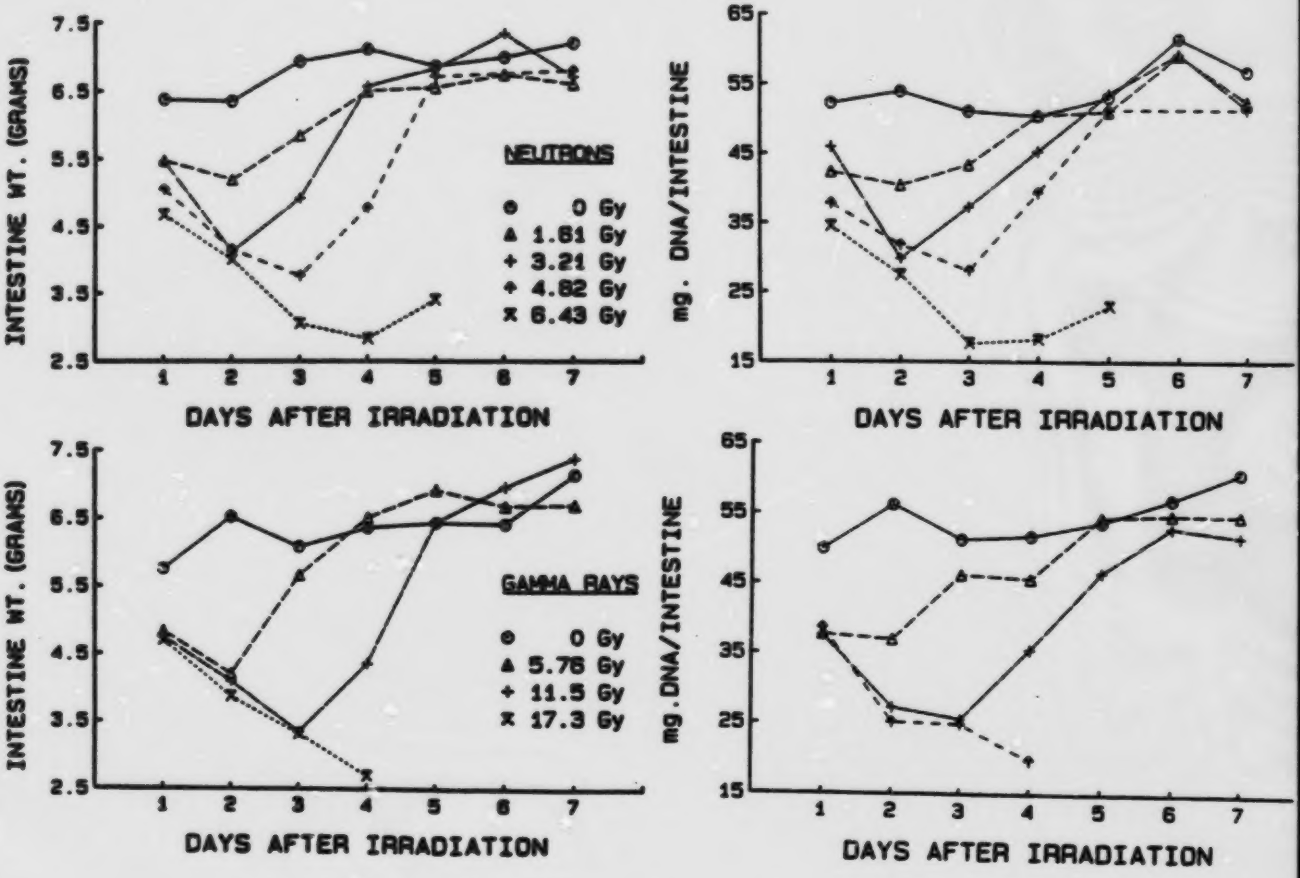

Figure 3. Weight and DNA content of small intestine as a function of time after various doses of neutrons or 137-Cs gamma rays. Each point represents the mean intestinal weight and DNA content of 8 to 16 rats. Standard errors range from 2 to 10 percent of the mean values.

Figure 3. Neutron doses (1.61, 3.21, and 4.82 Gy) and photon doses (5.76 and 11.5 Gy) which were sublethal for intestinal death resulted in a dose-dependent decrease in intestinal weight and DNA content. Depending on the dose, the maximum decrease in intestinal weight and DNA content occurred 2 to 3 days post exposure, with recovery to control values within 5 days. Doses of 6.43 Gy of neutrons and 17.3 Gy of gamma rays, which were 100% lethal within 5 days, caused greater mucosal breakdown and there was little or no recovery evident.

The results of microbiological analysis of cardiac blood and liver samples for sepsis are shown in Table 1. There was a low incidence of sepsis (7/115) detected in control animals which was probably due to contamination while obtaining the samples. The important finding is that during the first 5 days after irradiation, a period when mucosal breakdown is maximal, sepsis in the liver or blood was a rare event. This was also true of samples taken at autopsy from animals that had recently succumbed to intestinal injury. A similar result was also observed for portal blood samples obtained from 17.3 Gy gamma irradiated animals, hence, eliminating the possibility that the liver was sequestering and destroying bacteria released from the damaged gut. There was, however, indication of an increased incidence of sepsis after 5 days in 4.82 Gy neutron irradiated and 11.5 Gy gamma irradiated animals which probably was associated with developing hematopoietic injury. The organisms responsible for sepsis in these animals are indicated in Table 2. They were primarily *E. coli* and *Proteus mirabilis*. Most of the infections were caused by a single organism. All of the organisms isolated are normally found in the bacterial flora of the gastrointestinal tract of the rat.

Livers were also examined for the presence of endotoxin using both the LAL test and, in some animals, by injection of liver homogenate into mice sensitized to endotoxin by actinomycin D. Figure 4 shows the sensitivity of these two techniques. These data were generated by adding *E. coli* endotoxin standards to liver homogenates prepared from non-irradiated animals. The results show that the LAL test was more sensitive than the actinomycin D sensitized mice test, but both tests could detect endotoxin in nanogram amounts in the liver, which is a factor of a least 1,000 lower than the lethal levels for these rats (unpublished data). Among the animals listed in Table 1 which received doses of 0 to 6.43 Gy of neutrons and 0 to 17.3 Gy of photons, all livers were negative for endotoxin for the first 7 days after exposure. This included some animals who died from intestinal radiation injury and whose livers were removed immediately after death. To rule out the possibility that the negative LAL tests were due to inhibitors present in irradiated liver homogenates, negative samples were spiked with 1 ug of *E. coli* endotoxin. These spiked samples gave a 100% positive LAL test after incubation for an additional hour. Similar LAL results were obtained for portal blood obtained from 17.3 Gy photon irradiated animals.

TABLE 1

Sepsis in Blood and/or Liver

Time after irradiation (days)	*Number of rats positive for sepsis/total*							
	Radiation dose (Gy) Neutrons					*Radiation dose (Gy) 137-Cs gamma rays*		
	0.00	*1.61*	*3.21*	*4.82*	*6.43*	*5.76*	*11.5*	*17.3*
1	2/16	0/8	1/8	0/8	0/8	1/8	1/8	0/8
2	1/24	0/16	0/16	0/16	1/8	1/8	1/8	0/8
3	1/16	0/8	0/8	1/8	0/8	0/8	1/8	0/8
4	0/16	1/8	1/8	1/8	0/10	0/8	1/8	2/8
5	2/16	0/8	0/8	1/8	2/5	0/8	1/8	d
6	0/11	0/8	2/8	nd	d	0/8	3/8	d
7	1/16	0/8	0/8	2/8	d	0/8	5/8	d

d = all animals were dead at this time

nd = not determined at this time

TABLE 2

Organisms Isolated from Liver and Blood

Organism	*Number of animals positive for bacteria*			
	Unirradiated	*Gamma rays*	*Neutrons*	*Total*
E. coli	3	6	6	15
Proteus mirabilis	1	3	4	8
Lactobacillus	1	1	1	3
Streptococcus	1	3	1	5
Staphylococcus	2	2	1	5
Aerococcus	0	0	1	1
Klebsiella pneumonia	0	1	0	1
Enterococci	1	2	0	3
Cornium pyrogenes	0	2	0	2
Morganella morganii	0	1	0	1

Photons: single infection = 14, double infection = 2, triple infection = 1.

Neutron: single infection = 12, double infection = 1.

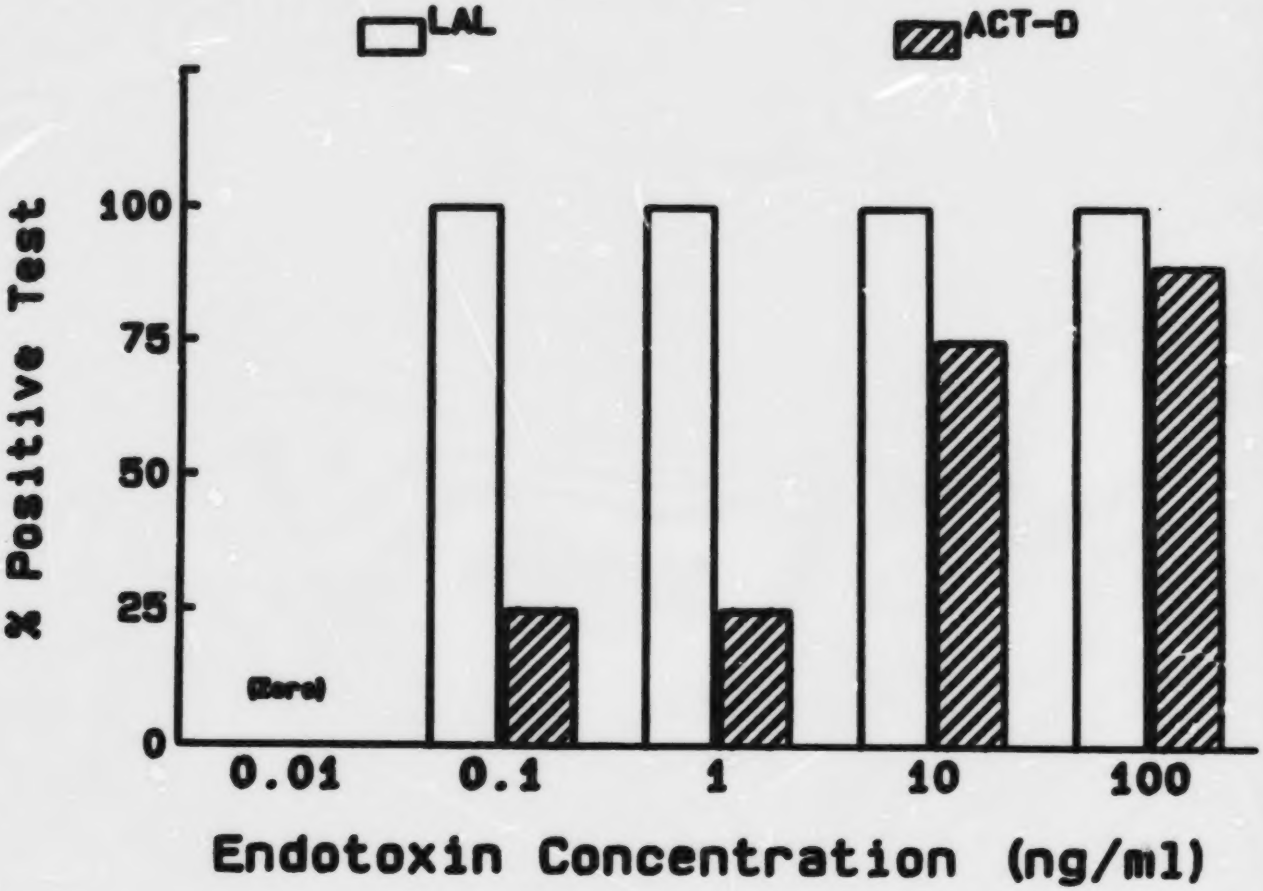

Figure 4. Endotoxin sensitivity of the Limulus Amebocyte Lysate test and Actinomycin-D sensitized mice test. These sensitivities were based on a minimum of 10 tests of endotoxin spiked liver homogenates prepared from livers of unirradiated rats.

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If endotoxin and bacterial infections are significant factors in gastrointestinal death, then increasing the host resistance to these agents by administration of sublethal doses of endotoxin before irradiation should result in increased survival and survival time. This has been shown to be true for radiation-induced hematopoietic death (73), in immunosuppressed mice (74), in experimental radiation hepatic injury (75), and in experimental gram negative infection and endotoxemia (76). To determine if increased resistance would also occur with intestinal radiation injury, 12 rats were preconditioned with increasing I.P., sublethal doses of E. coli endotoxin according to the schedule shown in Table 3. An equal number of animals were injected with endotoxin free water. The results given in Figure 5 show no beneficial effect of endotoxin treatment with respect to survival time or survival from intestinal death after 15.4 Gy whole-body gamma irradiation.

The incidence of excess fluid found in the small intestine as a function of dose and time after neutron and photon irradiations is shown in Table 4. Animals exposed to doses as low as 1.61 Gy of neutrons and 11.5 Gy of photons resulted in excess fluid in the intestine. Large amounts of fluid in the intestinal tract of all rats exposed to neutron doses greater than 3.21 Gy and photon doses of greater than 11.5 Gy occurred by 3 days after irradiation. At 4 days after irradiation large amounts of fluid in the intestine were still evident in most animals exposed to 4.82 and 6.43 Gy of neutrons and 11.5 and 17.3 Gy of photons. Only one animal exposed to 6.43 Gy of neutrons had excess fluid in the intestine 5 days after irrradiation.

The incidence of diarrhea as a function of time and dose after whole-body irradiation is shown in Figure 6 and Table 5. The ED-50 and 95% confidence limits for diarrhea are 4.88(4.78-4.98) Gy for neutrons and 10.3(9.72-10.9) Gy for gamma rays, yielding an RBE of 2.11(1.98-2.24). The median time for onset of diarrhea decreased from 3.9 days for neutron doses of 4.91 Gy and photon doses of 10.6 Gy to 3 days for neutron doses of 9.82 Gy and photon doses of 19.2 Gy. In animals not dying of intestinal death, diarrhea lasted 1 to 3 days.

The results presented in this study indicated that in SPF animals overt sepsis and endotoxemia do not play a significant role in the intestinal radiation syndrome. The presence of excess fluid in the intestine and diarrhea, but not bacteremia or endotoxemia, is correlated with the break down and recovery of the mucosa after irradiation. These results do not exclude, however, the possibility that bacterial action within the gut contributes to intestinal radiation death.

TABLE 3

Endotoxin Preconditioning Schedule

Day	*Treatment*
1	0.5 mg/kg body weight
3	1.0 mg/kg body weight
5	2.0 mg/kg body weight
7	4.0 mg/kg body weight
14	Whole-body irradiation

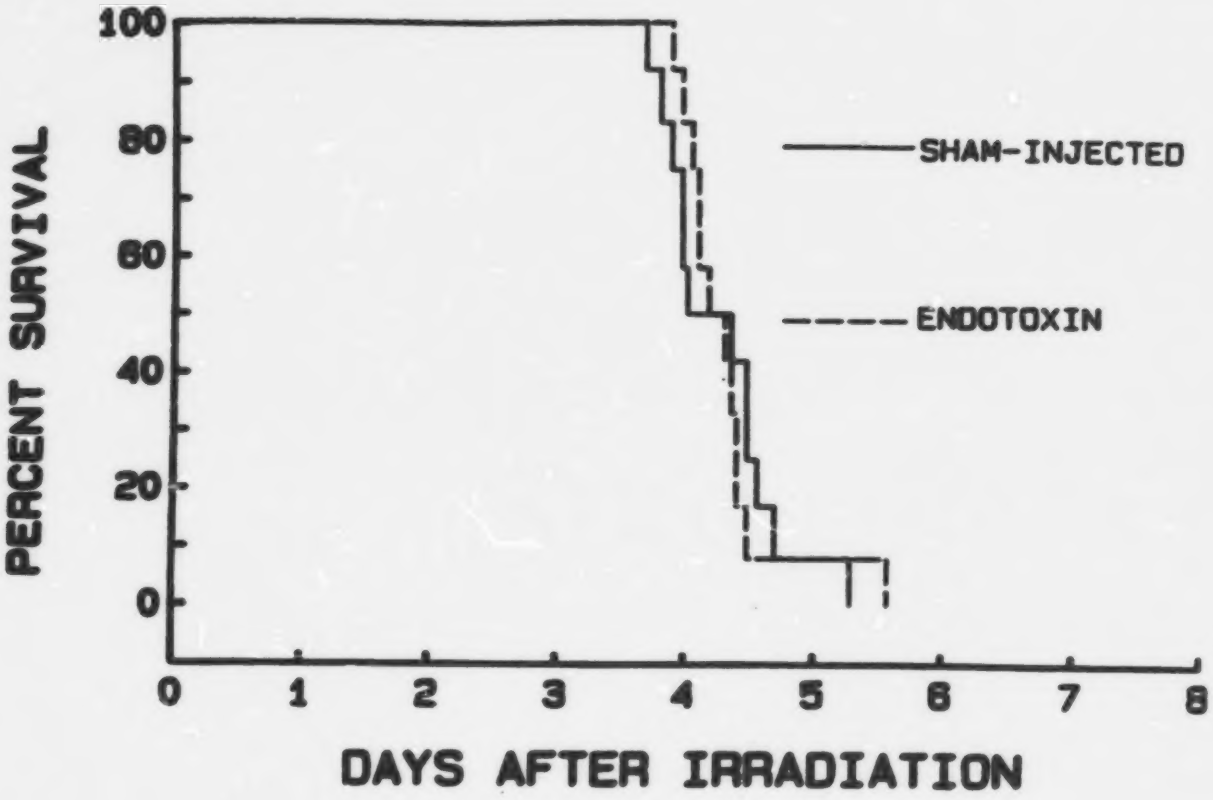

Figure 5. Percent survival of animals following I.P. injection of sublethal doses of endotoxin or endotoxin free water prior to 17.3 Gy whole-body gamma irradiation.

TABLE 4

Excess Fluid in GI Tract After Irradiation

Time after irradiation (days)	*Number of rats with excess fluid in GI tract/total*							
	Radiation dose (Gy) Neutrons					*Radiation dose (Gy) 137-Cs gamma rays*		
	0.00	*1.61*	*3.21*	*4.82*	*6.43*	*5.76*	*11.5*	*17.3*
1	0/16	0/8	0/8	0/8	0/8	0/8	0/8	0/8
2	0/24	0/16	0/16	1/16	0/8	0/8	0/8	0/8
3	0/16	1/8	8/8	8/8	8/8	0/8	8/8	8/8
4	0/16	0/8	0/8	8/8	8/10	0/8	8/8	8/8
5	0/16	0/8	0/8	0/8	1/6	0/8	0/8	d
6	0/11	0/8	0/8	nd	d	0/8	0/8	d
7	0/16	0/8	0/8	0/8	d	0/8	0/8	d

d = all animals were dead at this time

nd = not determined at this time

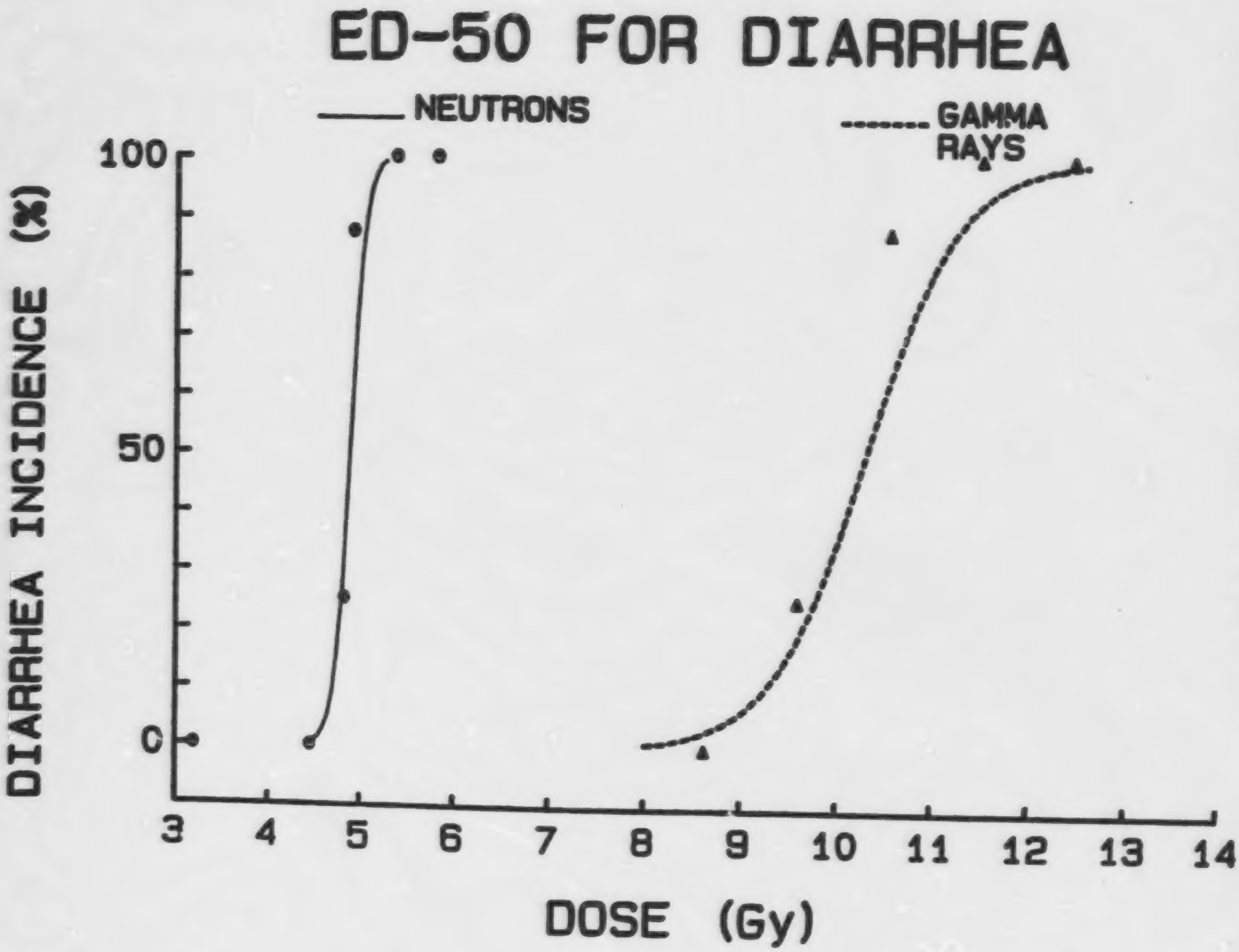

Figure 6. Dose response curves for the incidence of diarrhea following whole-body neutron or 137-Cs gamma ray irradiation. The curves represent a logistic function of the sigmoid curves that fit the data points with a Chi-square greater that 95%.

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TABLE 5

Median Time for Onset of Diarrhea

Neutrons		*137-Cs gamma rays*	
Dose (Gy)	*Median time (Days)*	*Dose (Gy)*	*Median time (Days)*
4.91	3.9	10.6	3.9
5.36	3.5	11.5	3.8
5.80	3.5	12.5	3.5
6.25	3.5	13.0	3.5
6.70	3.3	13.4	3.5
7.14	3.4	13.9	3.1
8.04	3.1	14.4	3.6
8.93	3.2	15.4	3.3
9.82	3.0	17.3	3.2
		19.2	3.0

Effect of Pseudomonas contamination or antibiotic decontamination of the GI tract on acute radiation lethality after neutron or gamma irradiation (Experiments 3 and 5 of Work Statement).

The organism used in this experiment, Pseudomonas aeruginosa, was proposed as the organism of interest by the AFRRI scientific staff and was obtained from Dr. Richard Walker of the National Naval Medical Center.

Figure 7 shows the change in the median survival time of 11.5 Gy 137-Cs gamma irradiated rats as a function of the number of Pseudomonas organisms orally intubated two days before irradiation. A dose of 11.5 Gy was chosen because it produces 5% and 100% mortality within 5 and 14 days after exposure, respectively. The results indicate that the administration of up to 10^6 organisms has little effect on survival time. Increasing the number of organisms intubated to 10^8 or 10^{10} resulted in significant reduction of survival time. Consequently, for subsequent experiments with Pseudomonas, 10^{10} organisms were selected for the oral dose to be administered to each rat.

To determine the concentration of Pseudomonas in various organs at the time of irradiation, 10 rats were sacrificed two days after oral intubation with 10^{10} Pseudomonas bacteria. Prior to sacrifice, blood was collected by cardiac puncture and cultured for the presence of Pseudomonas organisms. Immediately after death, liver, spleen, small intestine, and the cecum were aseptically removed and homogenized in sterile PBS and plated out on Cetrimide Selective Agar plates. No organisms were isolated from the spleen, liver or blood in these animals. Analysis of the small intestine and the cecum however, showed that each contained 10^3 to 10^5 Pseudomonas organisms. Essentially all the Pseudomonas in the intestines were associated with luminal contents since only 1% of these organisms were detected in homogenized intestine and cecum which had previously been rinsed to remove their contents.

Figure 8 shows the relationship between median survival time and neutron or gamma doses for Pseudomonas administered rats, conventional SPF rats, and antibiotic decontaminated rats. The results show a biphasic dose response curve for median survival time for all treatment groups. For conventional SPF animals, at cyclotron neutron doses greater than 6.7 Gy and gamma ray doses greater than 14.4 Gy, the median survival time was relatively constant at 4.2 to 4.5 days. Within the dose range of 4.9 to 6.7 Gy of cyclotron neutrons, and 9.6 to 14.4 Gy of photons, median survival time was inversely related to dose and varied from 12 to 4.6 days. Decontamination of the GI tract with antibiotics prior to irradiation significantly ($p<.05$) increased median survival time by 1 to 5 days in the range of neutron and gamma doses in which the median survival time was dose dependent. On the other hand, contamination of the GI tract with Pseudomonas organisms prior to irradiation significantly ($p<.05$) reduced the median survival time 1 to 5 days in this same dose range. The effects

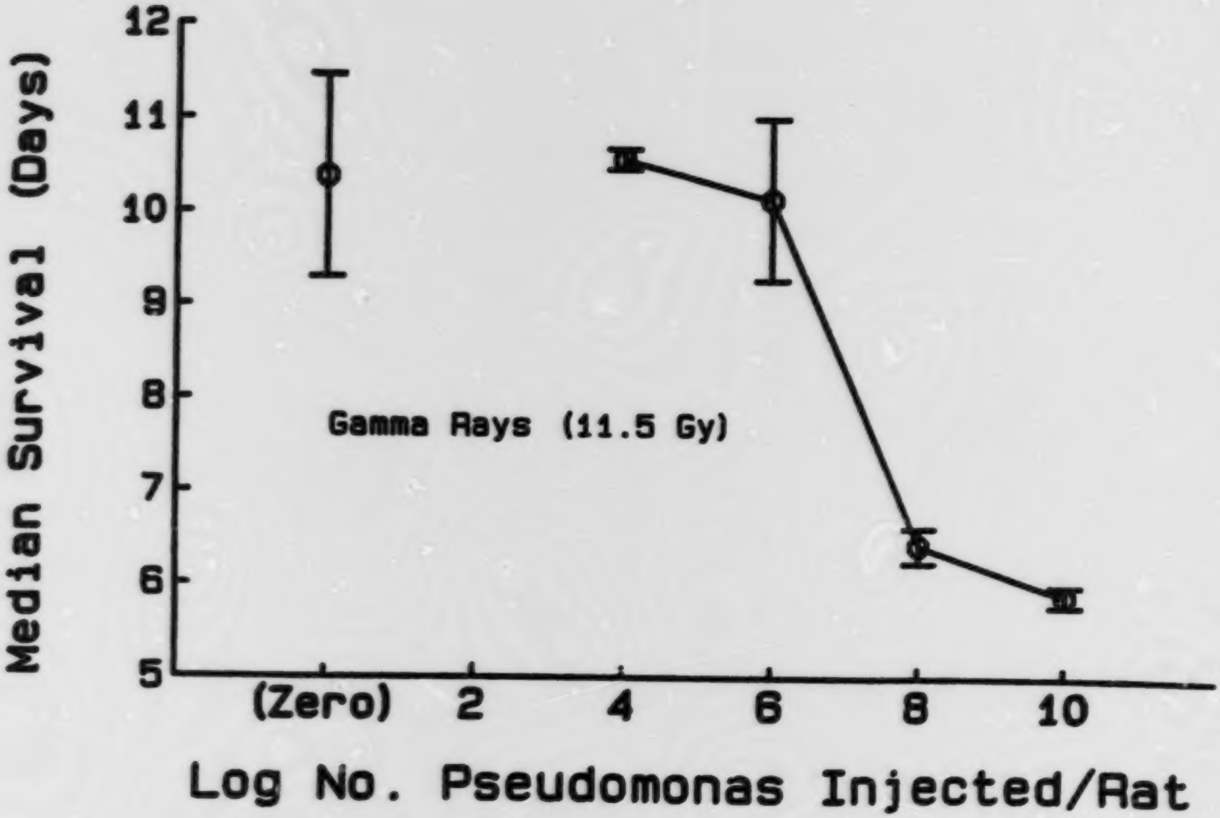

Figure 7. Survival time of 11.5 Gy 137-Cs gamma-irradiated animals as a function of the number of Pseudomonas organisms orally intubated into the stomach 2 days before irradiation. Each point represents the median survival time after irradiation of 10 animals infected with the indicated number of Pseudomonas organisms. Error bars represent standard errors.

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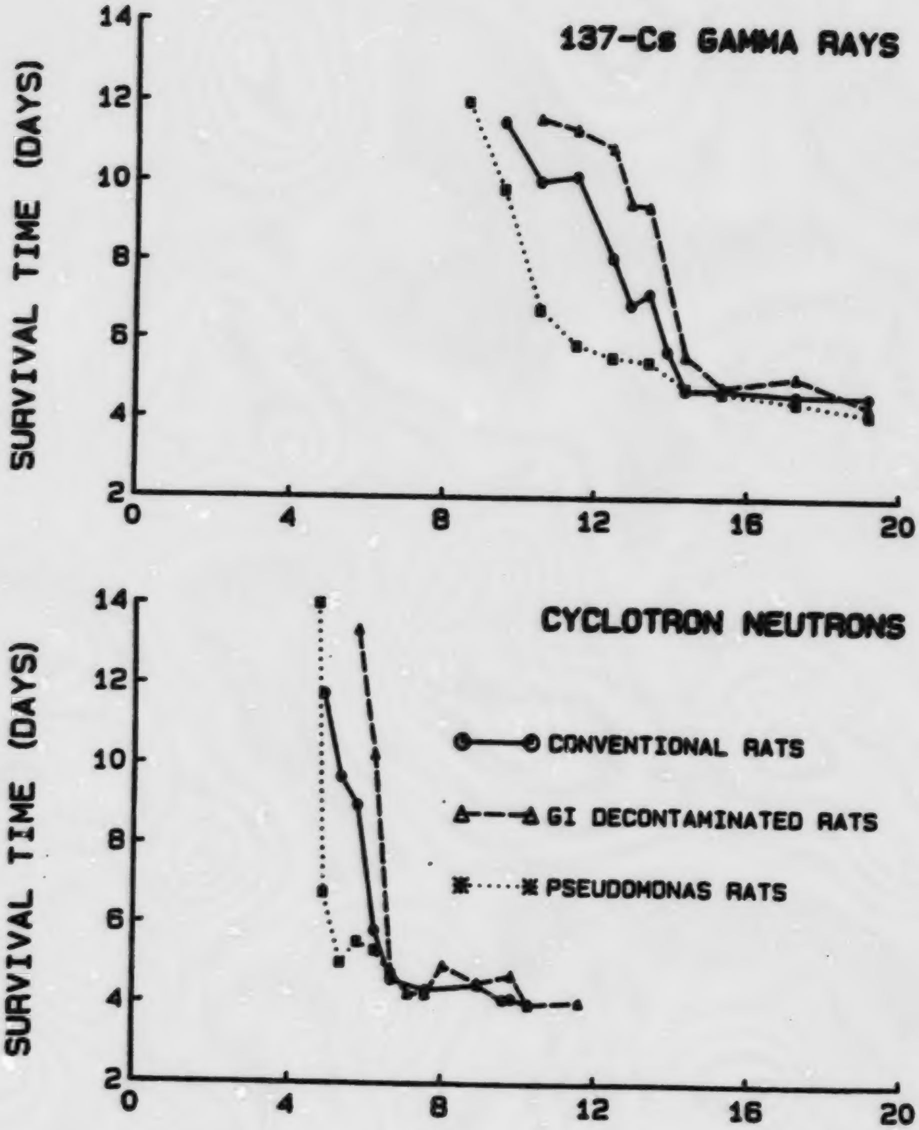

Figure 8. Survival time of untreated, GI decontaminated, and Pseudomonas infected animals after exposure to various doses of 137-Cs gamma rays and cyclotron neutrons. Each point represents the median survival time of 8 to 40 rats.

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of both decontamination of the GI tract and Pseudomonas contamination of the GI tract were negligible in the range of doses in which the median survival time is dose independent for conventional SPF rats.

Tables 6 and 7 give the LD 50/5 day, LD 50/30 day and Dose Modification Factor (DMF) for neutron and gamma irradiation of Pseudomonas contaminated, conventional SPF, and antibiotic decontaminated rats. In general, there appeared to be a slight increase in the radiation dose required to produce the LD 50/30 day and LD 50/5 day endpoints the cleaner the animals were at the time of irradiation (i.e., decontaminated > SPF-conventional > Pseudomonas treated). This increase in radioresistance, as measured by LD 50/30 day, was approximately 1 Gy for GI tract decontaminated animals as compared to contaminated animals (Table 1). This difference was only statistically significant for cyclotron neutron irradiated animals. The DMF for both Pseudomonas contamination and antibiotic decontamination of the GI tract was near unity for all types of radiation for both endpoints. The cyclotron neutron RBE for hematopoietic and intestinal death was 1.8 and 2.3, respectively.

Figure 9 shows terminal, total bacteria concentrations in the liver as a function of cyclotron neutron and 137-Cs gamma doses delivered to Pseudomonas contaminated rats. The bacteria in the liver at the time of death, as a result of irradiation, were mostly Pseudomonas (> 50%). At the lowest gamma dose that produced lethality (9 to 10 Gy) the bacteria concentrations were relatively low. Examination of these animals at autopsy showed internal hemorrhaging and development of ecchymosis on the feet and ears. This indicates that the mechanism of death was probably due to internal hemorrhaging. Bacterial concentrations were highest in the middle dose range (i.e., 10 to 14 Gy). Animals dying from gamma ray doses larger than 14 Gy had very low liver bacterial concentrations. A similar pattern was seen after neutron exposures except the maximum bacterial concentrations in the liver were usually lower than those occurring after gamma ray exposure.

Figure 10 shows the total liver bacteria concentrations of Pseudomonas contaminated rats at the time of sacrifice or the time of death after irradiation with doses from 3.8 to 19.2 Gy of 137-Cs gamma rays. In the groups of five animals exposed to either 19.2 Gy, 17.3 Gy, 15.4 Gy, or 14.4 Gy and sacrificed one, two or three days after exposure, there were no detectable bacteria in the livers. The highest bacterial concentrations in the livers occurred in animals dying 5 to 8 days after irradiation. In all the irradiated animals that survived 30 days after irradiation and were sacrificed, no bacteria were found in the livers. Figure 11 shows a similar pattern in the same animals for the percentage of livers tested positive for endotoxin.

The ED-50 and median time of onset of diarrhea in conventional, decontaminated, and Pseudomonas contaminated rats

TABLE 6

LD 50/5 DAY OF PSEUDOMONAS INFECTED GI, CONVENTIONAL, AND GI DECONTAMINATED RATS

Treatment	Radiation	LD 50/5 day (Gy)	DMF
Pseudomonas	^{137}Cs γ rays	13.7 (13.1-14.3)	0.97 (0.91-1.03)
Pseudomonas	C-Neutrons	5.85 (5.11-6.69)	0.94 (0.82-1.06)
Conventional	^{137}Cs γ rays	14.1 (13.4-14.7)	-
Conventional	C-Neutrons	6.25 (6.10-6.40)	-
Decontaminated	^{137}Cs γ rays	15.0 (13.9-16.2)	1.06 (0.97-1.15)
Decontaminated	C-Neutrons	7.40 (6.79-8.09)	1.18 (1.07-1.29)

DMF = Dose Modification Factor = (LD 50/5 day Treated)/(LD 50/5 day Conventional) for the same radiation type. "Treated" animals are Pseudomonas contaminated or GI decontaminated animals.

C-Neutrons = Cyclotron produced neutrons.

Values in parentheses are 95% confidence limits.

TABLE 7

LD 50/30 DAY OF PSEUDOMONAS INFECTED GI, CONVENTIONAL, AND GI DECONTAMINATED RATS

Treatment	Radiation	LD 50/30 day (Gy)	DMF
Pseudomonas	^{137}Cs γ rays	8.50 (7.96-9.23)	0.97 (0.87-1.09)
Pseudomonas	C-Neutrons	4.60 (4.30-4.92)	0.94 (0.85-1.04)
Conventional	^{137}Cs γ rays	8.75 (8.04-9.50)	-
Conventional	C-Neutrons	4.90 (4.56-5.27)	-
Decontaminated	^{137}Cs γ rays	9.60 (8.90-10.4)	1.10 (0.98-1.23)
Decontaminated	C-Neutrons	5.65 (5.32-6.00)	1.15 (1.04-1.26)

DMF = Dose Modification Factor = (LD 50/30 day Treated)/(LD 50/30 day Conventional) for the same radiation type. "Treated" animals are Pseudomonas contaminated or GI decontaminated animals.

C-Neutrons = Cyclotron produced neutrons.

Values in parentheses are 95% confidence limits.

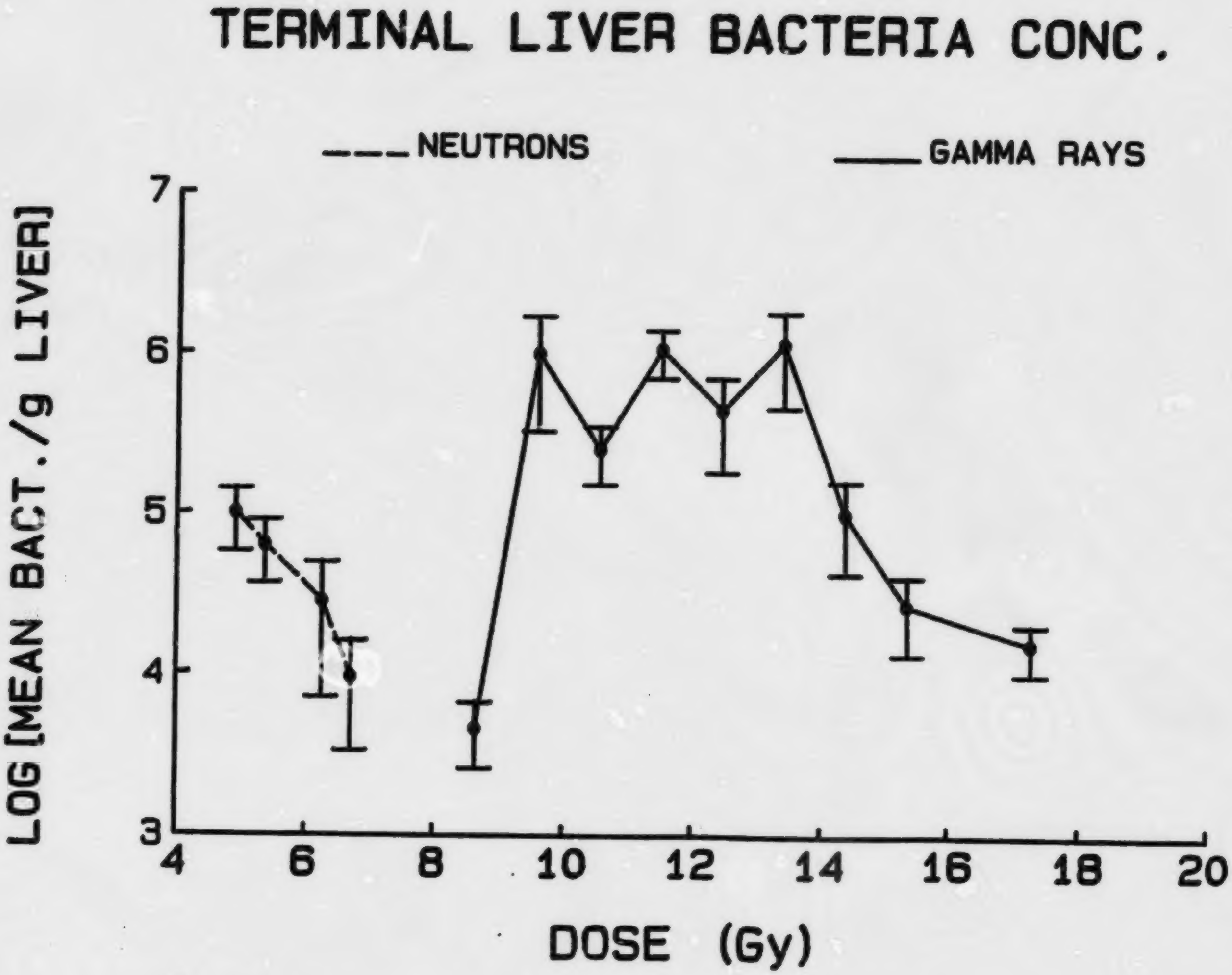

Figure 9. Liver bacteria concentrations at the time of death after exposure of Pseudomonas infected rats to graded doses of cyclotron neutrons and 137-Cs gamma rays. The number of animals used for each dose determination varied from 6 to 20 animals. Error bars represent standard errors.

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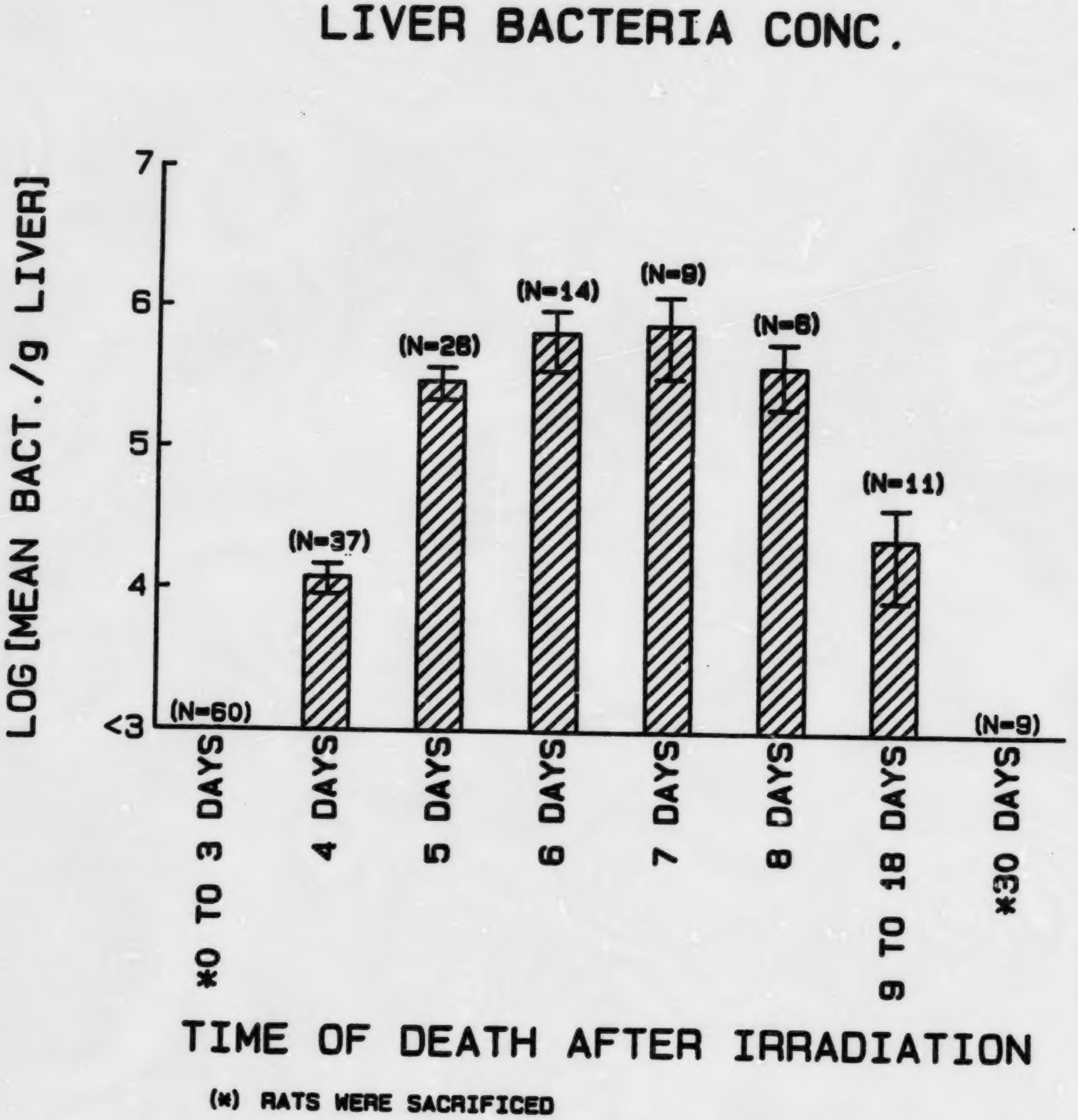

Figure 10. Liver bacteria concentrations at the time of death or time of sacrifice after exposure to 137-Cs gamma rays. The value in parenthesis at the top of each bar represents the number of animals assayed for liver bacteria concentration at the indicated time.

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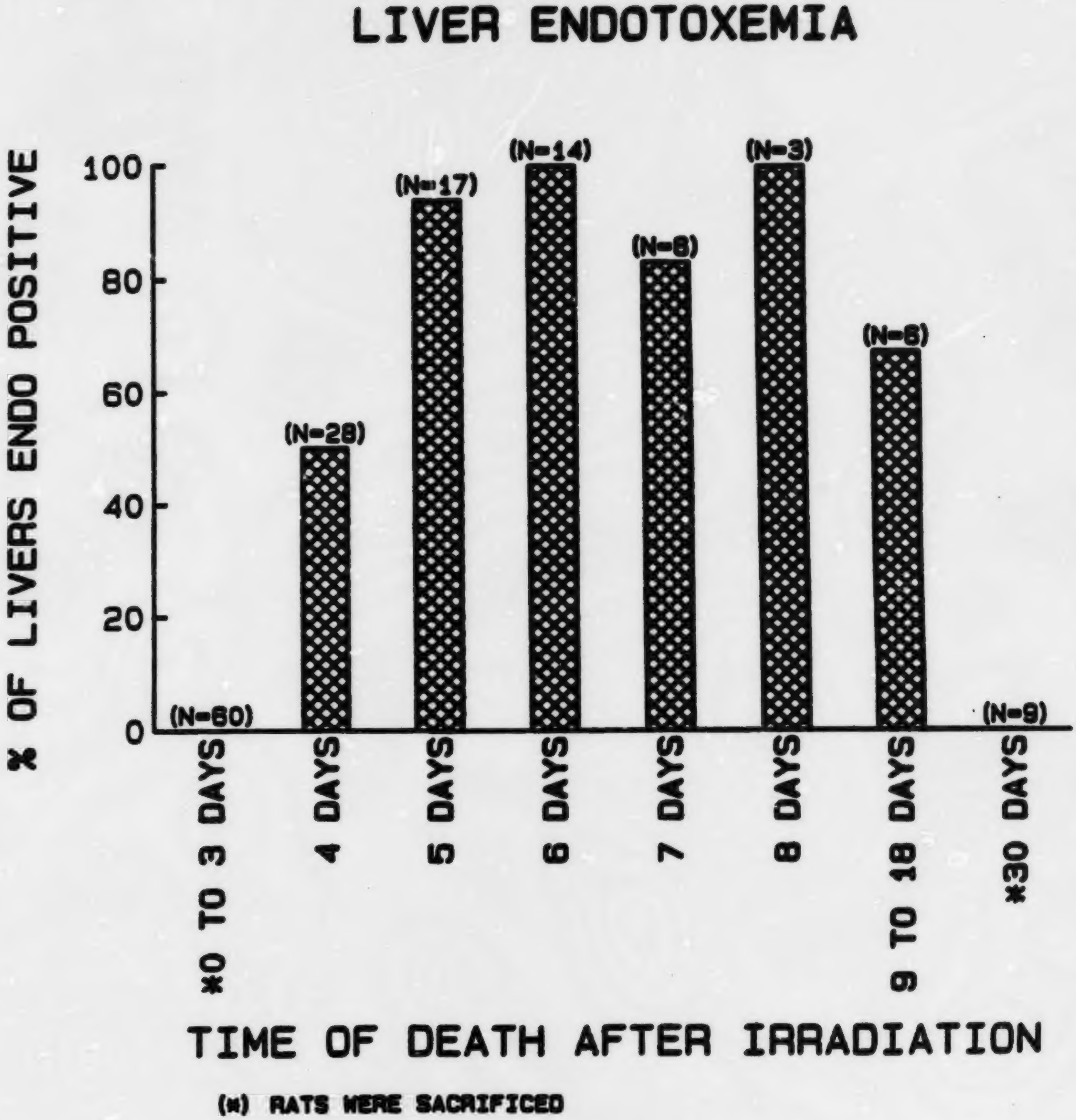

Figure 11. Liver endotoxemia at the time of death or time of sacrifice after exposure to 137-Cs gamma rays. The results are from the same group of animals presented in Figure 10. The value in parenthesis at the top of each bar represents the number of animals analyzed for endotoxin at the indicated time.

TABLE 8

Median Time for Onset of Diarrhea after Cyclotron-Neutron and 137-Cs Gamma Irradiation

Neutron Radiation dose (Gy)	(Days) Pseudomonas	Conventional	Decontaminated
4.91	*	3.9	
5.36	*	3.5	3.8
5.80	3.7	3.5	3.8
6.25	4.1	3.5	3.6
6.70	3.7	3.3	3.8
7.14		3.4	3.2
8.64		3.1	3.2
8.93		3.2	3.3
9.82		3.0	3.4
137-Cs Gamma Radiation dose			
10.6	*	3.9	*
11.5	*	3.8	*
12.5	4.3	3.5	3.3
13.0		3.5	3.6
13.4	3.9	3.5	3.3
13.9		3.1	3.1
14.4	3.6	3.6	3.0
15.4	3.4	3.3	2.8
17.3	3.5	3.2	3.0
19.2	3.4	3.0	2.7

*Less than 50% of the animals developed diarrhea.

TABLE 9

Effects of Antibiotic Decontamination and Pseudomonas Contamination of the GI Tract on the ED_{50} for Producing Diarrhea

Radiation	ED_{50} (and 95% confidence limits (Gy)		
	Conventional	Decontaminated	Pseudomonas
Gamma	10.3 (9.72-10.9)	11.6 (10.8-12.3)	11.7 (10.6-12.9)
Neutron	4.88 (4.78-4.98)	5.29 (4.72-5.92)	5.40 (4.82-6.04)
RBE	2.11	2.19	2.16

are shown in Tables 8 and 9. The results demonscrate that the presence or absence of normal enteric bacteria, or a pathogenic organism (Pseudomonas), in the intestinal tract has minimal effect on the diarrhea response after irradiation.

The results in the present study indicate that enteric bacteria do not play an important role in intestinal radiation death. Evidence for this is that decontamination or Pseudomonas contamination of the GI tract before irradiation had no effect on survival time in the pure gut death dose range where survival time is dose independent (Figure 8). Hence antimicrobial therapy will be largely ineffective in the treatment of individuals dying from pure intestinal radiation injury.

Postirradiation infection from endogenous enteric bacteria probably plays a significant role as a cause of death in a radiation dose range that results in death from a combination of intestinal and hematopoietic injuries (i.e., the "transitional dose range"). In this dose range antibiotic decontamination or Pseudomonas contamination of the GI tract has a significant effect on survival time (Figure 8) and on the highest degree of sepsis as measured by liver bacterial concentrations (Figure 9). Also, maximum liver bacterial concentrations and the highest incidence of endotoxemia were observed in animals dying 5 to 8 days post exposure (Figures 10 and 11) when there is an overlap of intestinal and hematopoietic injuries in these animals. Hence, antimicrobial therapy after irradiation to suppress infection will probably be effective in prolonging survival in this low dose range. This dose range is much smaller after neutron as compared to gamma ray exposures (Figure 8). Nevertheless, antimicrobial therapy will probably be more effective after neutron exposure within this narrow dose range due to less severe hematopoietic injury, resulting in a lower degree of sepsis requiring treatment (Figure 9).

The data in this study indicate that antimicrobial therapy will probably not prevent most of the acute deaths from exposure to ionizing radiation unless accompanied by procedures that result in regeneration of the intestine and bone marrow. The reason for this is that decontamination of the GI tract or Pseudomonas contamination of the intestinal flora had little effect on survival as measured by the LD 50/5 day or the LD 50/30 day (Tables 6 and 7), and as indicated by DMF values being close to 1.0 for these treatments. This is probably because antimicrobial therapy treats the consequences (i.e., sepsis from enteric organisms) rather than the cause of radiation death (i.e., cell depletion of the bone marrow and the intestinal mucosa).

Effect of bile-duct ligation on survival time after neutron and gamma irradiation of normal and antibiotically-treated animals. (Experiment 4 of Work Statement)

The effects of BDL on survival time in conventional and decontaminated rats after various doses of 137-Cs gamma rays and cyclotron produced neutrons are shown in Figure 12. BDL significantly prolonged survival by 1 to 2.5 days in the radiation dose range where survival time is relatively dose independent for both types of irradiation and the cause of death is primarily due to intestinal damage (2,77,79). BDL also prolonged survival of animals exposed to doses in which survival time is dose-dependent, i.e., where death is due to a combination of intestinal and hematopoietic injuries (2,77,79). At low gamma ray and cyclotron neutron doses, where survival time of non-BDL rats is 10 to 12 days and death is mainly due to hematopoietic injury, BDL had no effect, or in some instances decreased survival time. The shorter survival time due to bile duct ligation in rats irradiated with lower gamma ray doses (Figure 12) and dying of hematopoietic injury is attributed to the toxicity of prolonged ligation (80) and the subsequent impairment in the liver's ability to combat infection. In general, decontamination with BDL was more effective than BDL alone in prolonging survival but this effect diminished at high doses where death is primarily due to intestinal injury.

The prolongation of survival in BDL rats was accompanied by a delay in the onset of diarrhea and reduction in the percentage of animals that developed diarrhea prior to death. For example, in the predominantly gut death dose range where survival time is relatively independent of dose (i.e., cyclotron neutron doses >6.25 Gy and 137-Cs gamma ray doses >14.4 Gy) 221 out 223 conventional animals developed diarrhea with a median onset time of 3.1 days (2.9-3.2, 95% confidence interval) after irradiation. In comparison, 85 out 113 BDL animals developed diarrhea with a median onset time of 4.1 days (3.9-4.3, 95% confidence interval). In BDL, decontaminated rats diarrhea rarely developed before death. It should be emphasized, however, that BDL rats refused to drink antibiotic water after irradiation, thus they became severely dehydrated sooner than non-BDL irradiated rats.

In summary, the results obtained by combining bile duct ligation and decontamination treatments suggest that alimentary bacteria play a minor role in intestinal death produced both by gamma radiation and neutron radiation. However, for both types of radiation, at all the doses studied which produced primarily intestinal death (plateau region) bile duct ligation alone was more effective than decontamination alone in increasing survival time (see Figure 8). Part of the effect of decontamination when combined with bile duct ligation may be the result of an increase in survival time produced by the bile duct ligation. Bile duct ligation alone would allow time for overgrowth by bacteria due to developing hematopoietic injury, a response partially negated by decontamination of the GI tract.

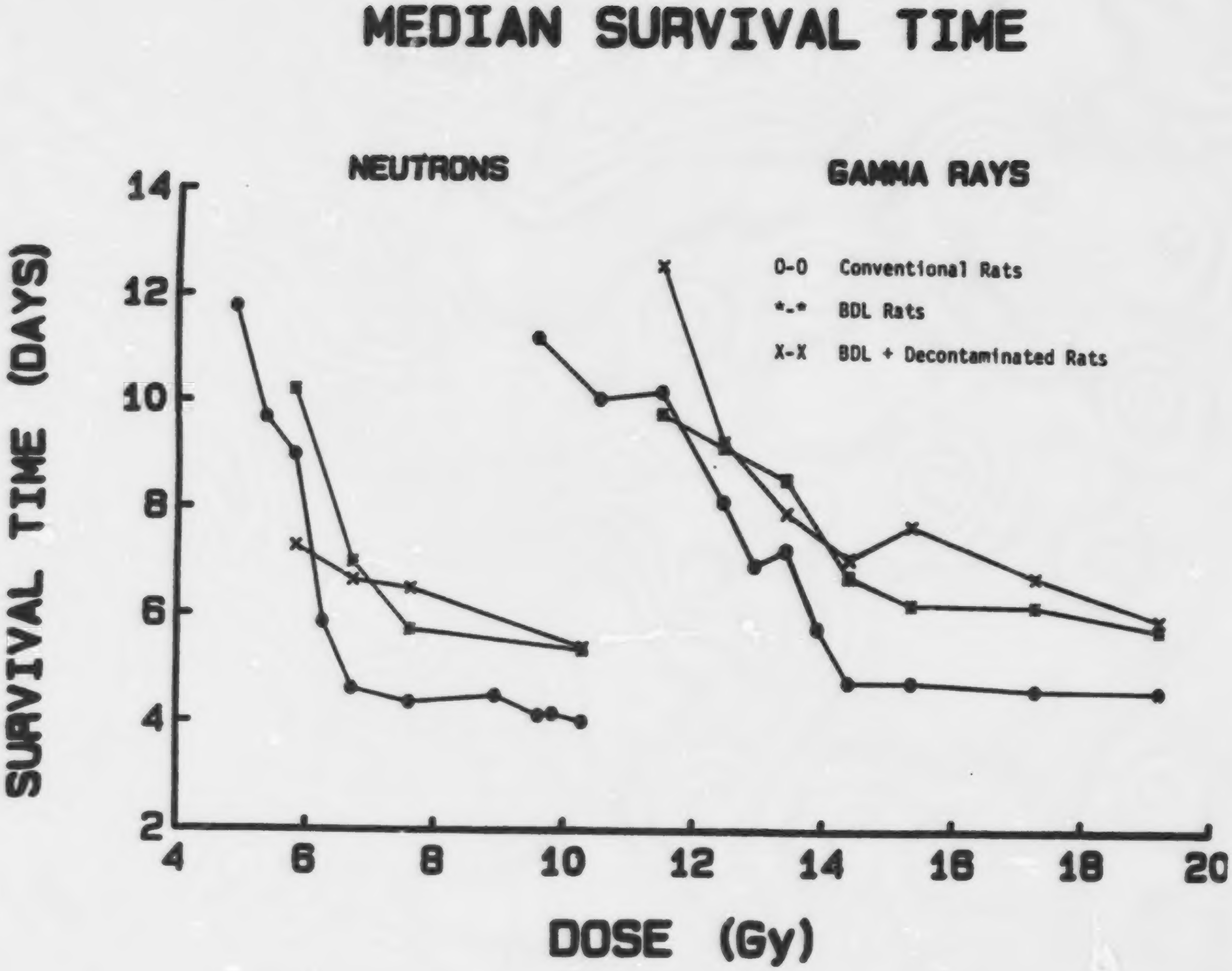

Figure 12. The effect of BDL on survival time of conventional and GI decontaminated rats after exposure to graded doses of cyclotron produced fast neutrons or 137-Cs gamma rays. Each experimental point represents the median survival time of at least 8 animals. Error bars are omitted for clarity. 95% confidence limits were 2 to 7% of the mean values.

Role of bile acids in acute intestinal radiation death (Substitute Experiment 9 of Work Statement, modification of contract DNA001-83-C-00009-P00003 dated 27 March 1984).

Figure 13 shows the results of an experiment designed to determine if the presence of bile acids in the small intestine negates the increased survival time and increased time for onset of diarrhea which has been shown to occur in BDL rats dying from lethal intestinal radiation injury (Figure 12).

In this experiment rats were surgically prepared with externally exposed cannulae which ran from the back of the neck to the lumen of the duodenum. Duodenal cannulated animals were bile duct ligated (BDL) or sham bile duct ligated (SBDL) one day post irradiation. Starting one day after irradiation, 4 to 5 cc of lactate- Ringer (LR) solution containing 0, 19, or 38 millimolar taurocholic acid (Sigma Chemical Co.) was injected into the small intestine via this cannula 3 times a day at 8 hour intervals by temporarily connecting a 5 cc syringe to the teflon needle on the back of the animals. Taurocholic acid was used because it is the primary bile acid secreted by the rat (30). The taurocholic acid concentrations of 19 and 38 mM were chosen because they encompass the range of bile acid concentration reported in the literature for the rat (39,81,82). LR solution or LR solution containing taurocholic acid was administered at eight hour intervals until the death of the animal to simulate the normal volume of bile flow into the intestine.

The median survival time of 6.7 days for BDL rats receiving LR alone (0 mM taurocholic acid) is significantly longer ($p=0.004$) than the median survival time of 4.1 days for the corresponding SBDL rats (Figure 13). This difference between median survival time of BDL and SBDL animals was decreased by addition of taurocholic acid to the LR solutions injected into the duodenum, primarily due to a reduction in survival time of the BDL animals. The median survival time of BDL and SBDL animals injected with 19 mM taurocholic acid in LR solution was 5.7 and 4.0 days, respectively. The median survival time of BDL rats injected with 38 mM taurocholic acid in LR solution was reduced to 3.9 days and was not statistically different from the median survival time of 3.8 days for similarly treated SBDL rats.

The influence of taurocholic acid injected into the intestine on median time for onset of diarrhea in these BDL and SBDL rats is shown in Figure 14. The onset of diarrhea in animals injected with LR solution alone (0 mM taurocholic acid) was significantly longer ($p<0.05$) in BDL animals than in SBDL animals. The addition of taurocholic acid to the LR solution injected into the duodenum brought on diarrhea slightly earlier in both SBDL and BDL irradiated rats. However, the relative difference in the median time for onset of diarrhea between the BDL and SBDL rats remained relatively constant as the taurocholic acid concentration was increased. The median time of onset of diarrhea for the BDL group of rats at 0, 19, and 38 mM taurocholic acid concentration was 4.4, 4.2, and 3.9 days,

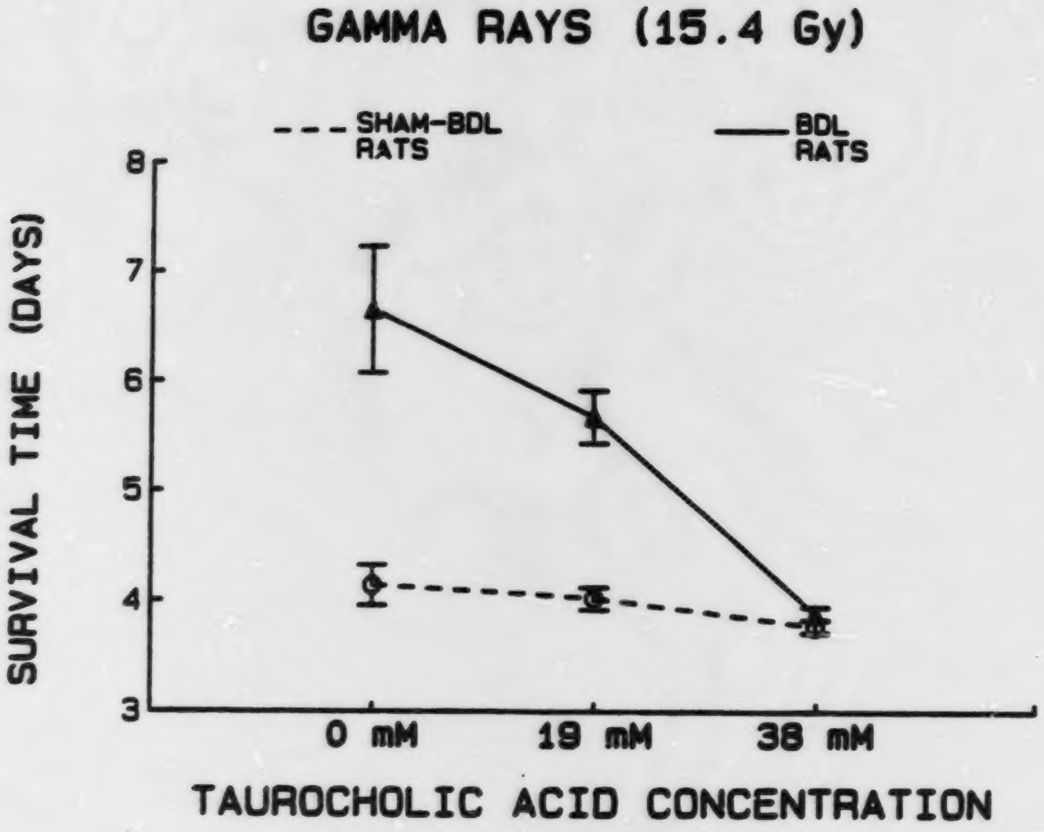

Figure 13. The effects of taurocholic acid injection into the duodenum on the survival time of BDL and SBDL 15.4 Gy whole body irradiated rats. Five cc of taurocholic acid at the indicated concentration in LR solution was injected into the lumen of the duodenum beginning 1 day post irradiation via a previously inserted cannula. Prior to starting taurocholic acid injections the animals were BDL or sham-BDL. Each point represents the median survival time of 7 to 18 animals. The bars represent 95% or higher confidence intervals.

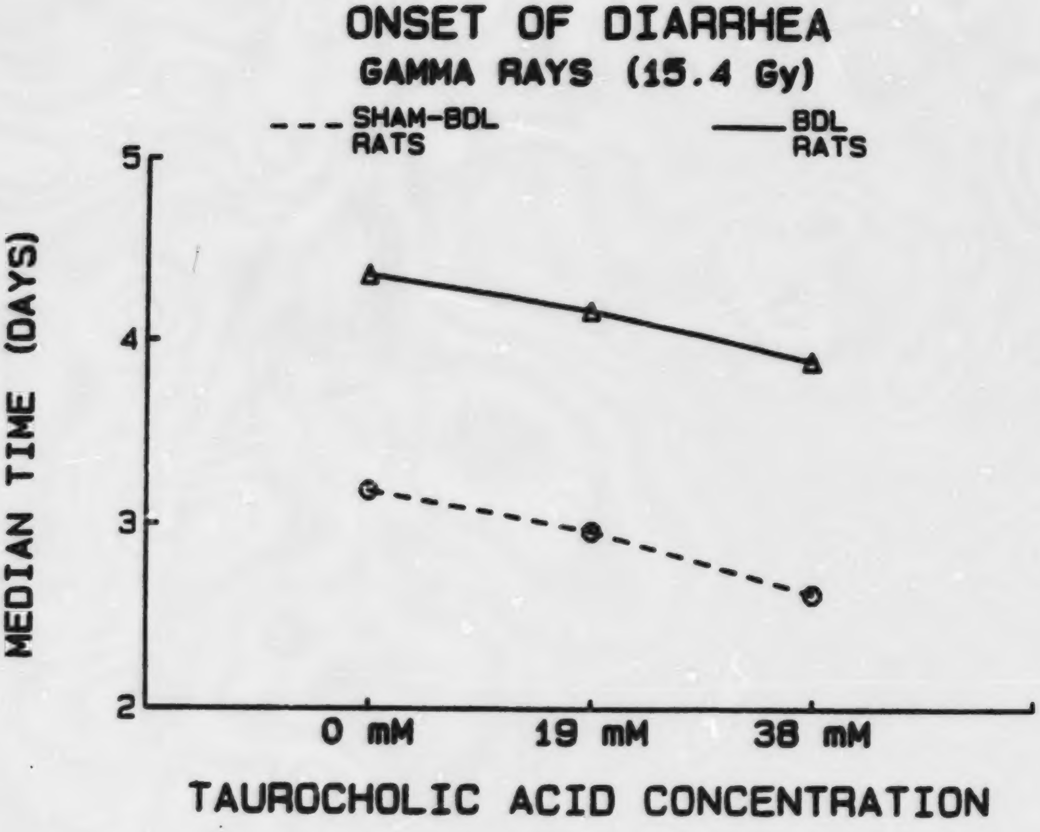

Figure 14. The effects of taurocholic acid injection into the duodenum on the median time for onset of diarrhea in irradiated BDL animals. The results are from the same group of animals presented in Figure 13.

respectively and the corresponding median time for onset of diarrhea for the SBDL rats was 3.2, 3.0, and 2.6 days.

Figure 15 shows the effects of injecting taurocholic acid into the intestine on the percentage of these animals which developed diarrhea prior to death. Nearly all (>92%) of the SBDL animals developed diarrhea whether or not taurocholic acid was injected into the intestine. In contrast, bile duct ligation reduced the percentage of animals (70%) that developed diarrhea prior to death and injection of taurocholic acid into the duodenum had no effect on this response.

From examination of the BDL irradiated animals which had received taurocholic acid, it became clear they were not exhibiting a typical intestinal radiation death. These animals had massive, bloody ascites which is not seen in animals dying with the intestinal radiation syndrome, with or without BDL. Histological examination of the livers showed inflammation around the blood vessels and bile ducts (Figure 16).

To determine if the reduction in survival time caused by taurocholic acid injection into bile duct ligated rats (Figure 13) may have been independent of the radiation injury, non-irradiated bile duct ligated and sham bile duct ligated rats were injected with taurocholic acid via the intestinal cannulae. The results of this experiment are given in Figure 17. When no taurocholic acid was injected into the small intestines of bile duct ligated rats, all animals survived beyond 7 days. However, injection of either 19 mM or 38 mM solutions of taurocholic acid (4 ml three times daily) caused significant mortality within seven days. This occurred only if the bile duct was ligated. There was 100% survival at 7 days in sham bile duct ligated animals which received 38 mM taurocholic acid. The possible cause of these effects is the increase in systemic bile acids caused by a combination of bile duct ligation and injection of exogenous taurocholic acid. Therefore, the decrease in survival time due to taurocholic acid seen in Figure 9 may be due to the toxicity of the taurocholic acid which negates the protective effect of bile duct ligation rather than an interaction between bile acid and radiation injury.

From these data one cannot determine whether bile acids are important factors in acute intestinal radiation death. Therefore, a study was made of the effectiveness of exteriorizing the bile by cannulation (choledochostomy) so that no bile entered the small intestine. If bile acids are important in the acute intestinal radiation syndrome, then choledochostomy to eliminate bile acids from the radiation damaged intestine, combined with duodenal injection of LR to compensate for the fluid lost by bile drainage, should prolong survival time similar to that produce by BDL.

The median survival time and diarrhea incidence of 15.4 Gy irradiated rats with a choledochostomy are given in Table 10. The results show that choledochostomy and duodenal injection of

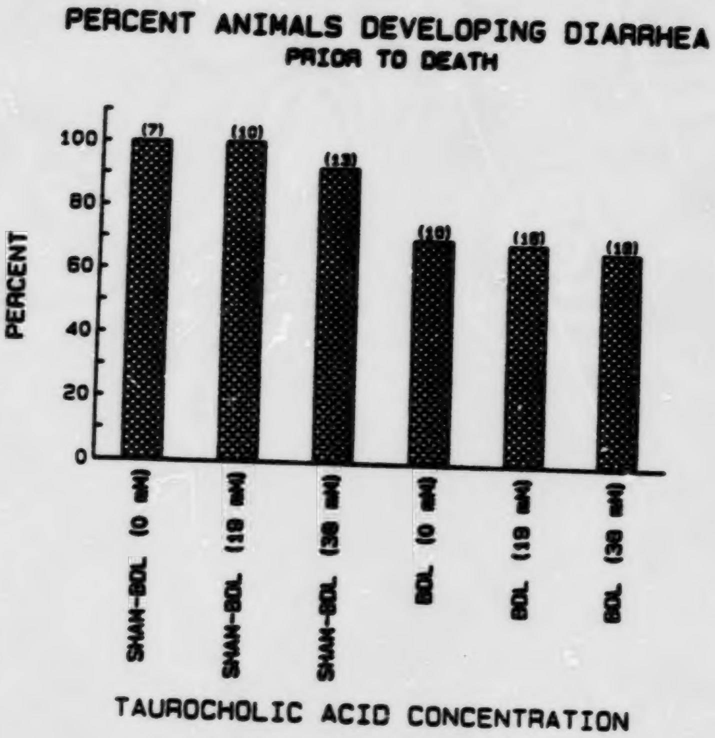

Figure 15. The effects of taurocholic acid injection into the duodenum on the percentage of animals developing diarrhea prior to death. The results are from the same animals presented in Figures 13 and 14. The value in parenthesis at the top of each bar represents the number of animals in each treatment group.

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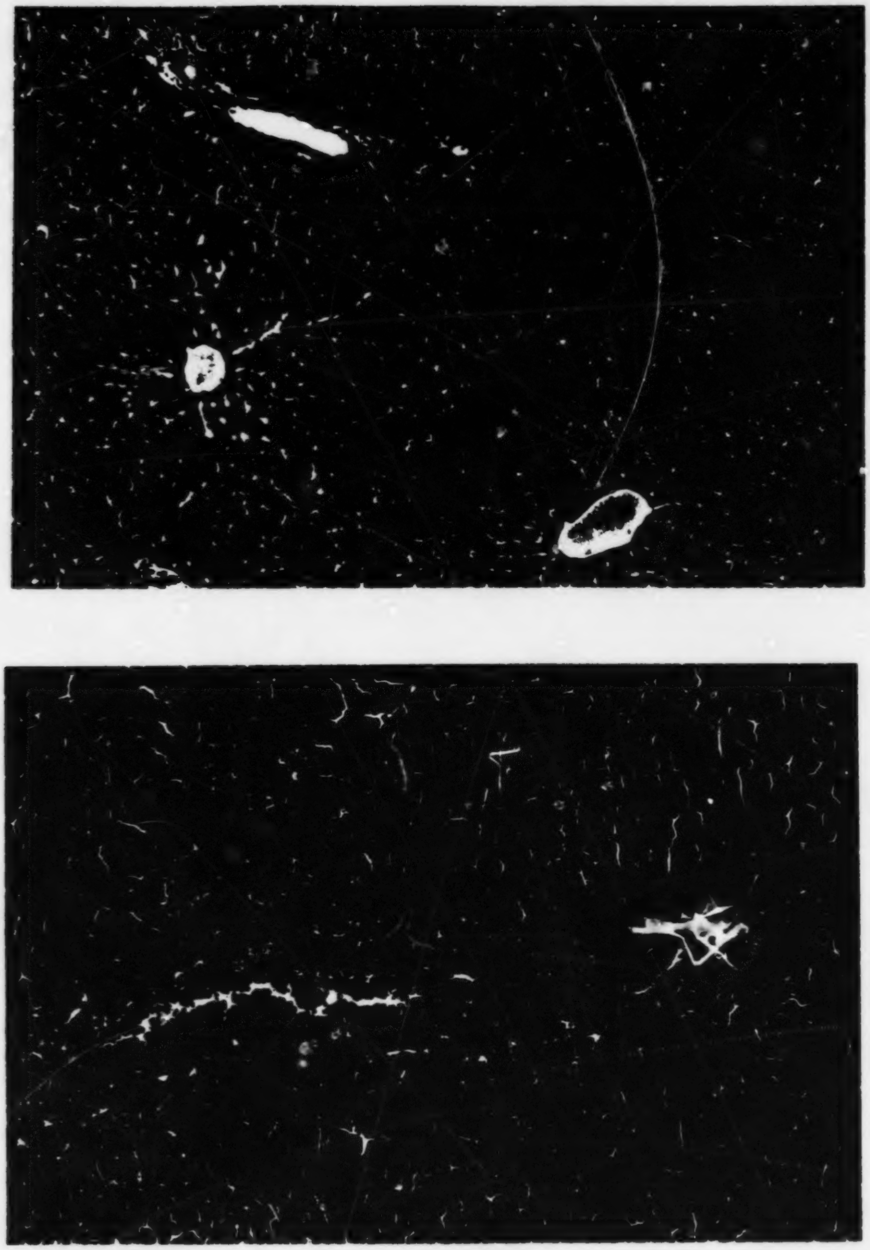

Figure 16. H & E stained sections of liver obtained from nonirradiated BDL and sham-BDL animals duodenally injected with 15cc/day of LR solution containing 38 mM taurocholic acid. Top: periportal region from sham-BDL animals showing no inflammation. Liver specimen was obtained from a sacrificed animal 7 days after beginning treatment with 38 mM taurocholic acid. Bottom: periportal region from BDL animal showing marked inflammation. Liver sample was obtained at death (5 days after beginning treatment with 38mM taurocholic acid).

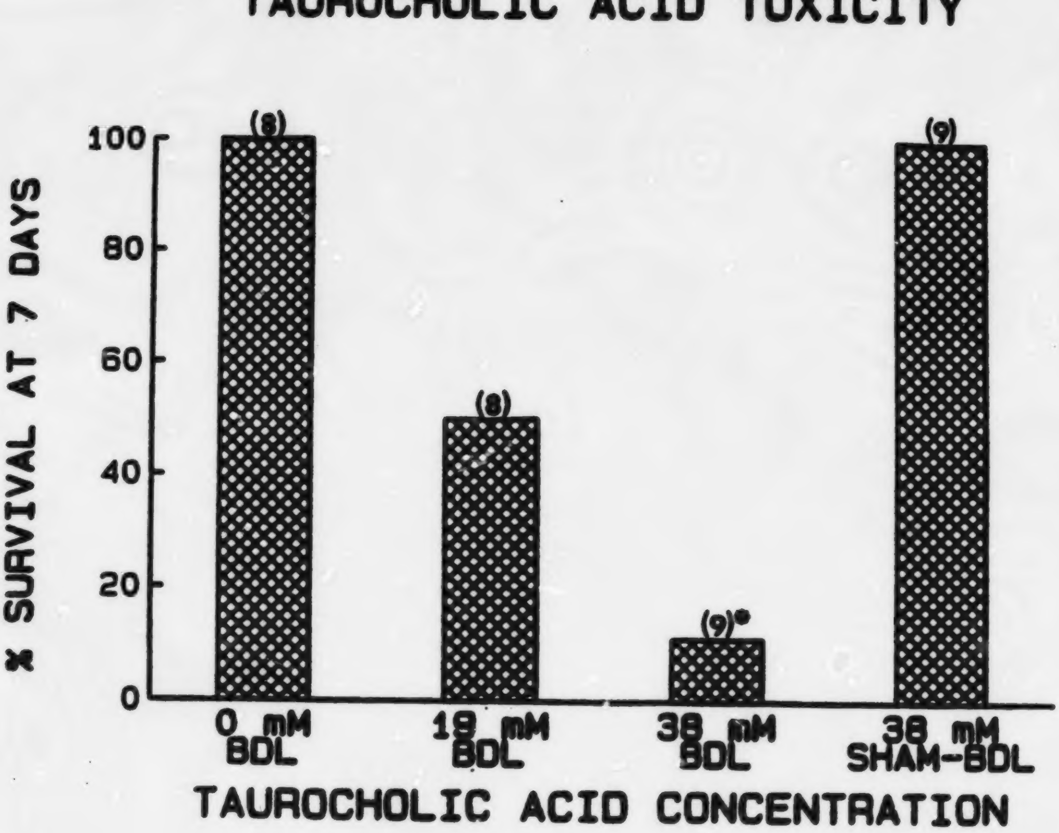

Figure 17. Taurocholic acid toxicity in nonirradiated BDL animals. Animals were BDL or sham-BDL seven days after insertion of cannula into the lumen of the duodenum. Following ligation or sham ligation of the bile duct, 15cc/day of LR solution containing the indicated concentration of taurocholic acid was injected into the duodenum via a cannula inserted into the lumen of the duodenum. The number in parenthesis above the bars represents the number of animals per treatment group. An asterisk above the bar indicates a significant difference ($p<0.05$) as compared to either the 0 mM BDL or the 38 mM sham-BDL groups based on confidence intervals.

TABLE 10

Effect of Elimination of Bile from the Intestine on Median Survival Time and Diarrhea Incidence of 15.4 Gy Whole-Body Irradiated Rats

Treatment	No. of Rats	% Diarrhea	Median Survival Time[1]
Irradiated Control	38	97	4.6(4.4-4.9)
Irradiated with Fluid Replacement (15 cc/day Duodenal Injection)	8	100	4.2(3.9-4.6)
Irradiated and Bile Flow Exteriorized (choledochostomy) with No Fluid Replacement	19	0	3.5(3.3-3.7)
Irradiated and Bile Flow Exteriorized (choledochostomy) with Fluid Replacement (15 cc/day Duodenal Injection)	10	100	4.5(4.3-5.0)
Irradiated and Bile Duct Ligated	22	77	6.1(5.7-6.8)

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1. Time in Days after Irradiation.

2. Values in Parentheses are 95% or Greater Confidence Limits.

LR had no effect on survival time of irradiated rats whereas BDL irradiated rats had a significantly (P<.05) longer survival time than untreated or bile drained irradiated animals. The shortest survival time was seen in the 15.4 Gy irradiated choledochostomized animals that received no fluid replacement. This is evidence against the hypothesis that the mucous depleting action of bile acids on the irradiated intestine results in increased leakage of fluid and electrolytes into the intestinal lumen, inducing lethal diarrhea.

Without fluid replacement, no diarrhea was evident in any of the 19 choledochostomized irradiated animals before death. However, in 10 irradiated animals with choledochostomy which received duodenal injections of LR (15 ml/day), all developed diarrhea before death. It is hypothesized that the absence of diarrhea in irradiated bile drained animals without fluid replacement is not due to the absence of bile acids in the intestinal lumen as postulated by Sullivan (30-32), but rather is due to the fact that bile is an important source (up to 15 ml/day) of fluid and electrolytes. This source is eliminated by exteriorizing the bile flow. Moreover, the short survival time post irradiation of choledochostomized animals without fluid therapy probably preempts the development of diarrhea. For example, in 15.4 Gy irradiated animals in which bile fluid is prevented from entering the intestine by BDL, 95% of the diarrhea occurred 3.9 to 4.3 days after irradiation, a time at which more than 95% of the 15.4 Gy irradiated choledochostomized rats without fluid therapy already had died (Table 10).

Table 11 shows the influence of duodenal injection of cholestyramine (1 or 3 grams/day) on survival time and diarrhea when the cholestyramine was given after 16 Gy gamma irradiation. All animals in each treatment group died and the median survival times were not statistically different among the cholestyramine administered and untreated groups. Onset of diarrhea was delayed only in the cholestyramine group which received 3 grams per day of the resin. Autopsies of these animals showed that this delay in diarrhea was due to the large bowel being plugged with resin. The proximal portions of the GI tract (cecum, small intestine, and stomach) were grossly distended with fluid.

The observation that post irradiation duodenal injection of cholestyramine to bind bile acids does not affect diarrhea incidence, survival or survival time (Table 11) further supports our contention that bile acids are not a critical factor in acute intestinal radiation death.

Administration of antibiotics and saline into the GI tract and resultant effects on survival time after irradiation (Experiment 10 of Work Statement).

The effects of fluid replacement therapy on survival time of choledochostomized nonirradiated animals are shown in Table 12. In these animals in which the bile was exteriorized, fluid and

TABLE 11

Effect of Post-Irradiation (16 Gy Whole-Body) Duodenal Injection of Cholestyramine on Diarrhea and Survival Time

Treatment	No. of Rats	% Diarrhea	Median Diarrhea Onset Time (days)[1]	Median Survival Time (days)[1]
None	16	94	2.9(2.7-3.7)	4.4(3.9-4.7)
Cholestyramine (1 mg/day)	12	100	2.7(2.6-3.2)	4.2(3.7-4.7)
Cholestyramine (3 mg/day)	8	88	4.1(3.7-4.7)	4.4(4.0-4.8)

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1. Values in Parentheses are 95 % or Greater Confidence Limits.

TABLE 12

Survival Time of Unirradiated Bile-drained Rats vs that of 15.4 Gy Whole-Body Irradiated Rats Without Bile Drainage

Treatment	No. of Rats	Median Survival Time[1,2]
15.4 Gy Whole-Body Irradiated (Control)	38	4.6(4.4-4.9)
Unirradiated, Bile Flow Exteriorized	10	4.4(3.8-5.5)
Unirradiated, Bile Flow Exteriorized, with Fluid Replacement (15 cc/day Duodenal Injection)	10	No Deaths
Unirradiated, Bile Flow Exteriorized, with Fluid Replacement (15 cc/day Peritoneal Injection)	10	No Deaths

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1. Time in Days after Irradiation or Bile Duct Cannulation.

2. Values in Parentheses are 95% or Greater Confidence Limits.

electrolyte were administered by injecting LR into the peritoneal cavity or into the lumen of the duodenum via a second cannula. Exteriorizing the bile in nonirradiated animals receiving no fluid and electrolyte replacement was 100% lethal with a median survival time of 4.6 days. This survival time is similar to that seen in 15.4 Gy irradiated rats dying from acute intestinal injury. Fluid and electrolyte replacement either by duodenal or peritoneal injections prevented death in bile exteriorized nonirradiated rats.

Table 13 shows the results of experiments designed to determine if various forms of fluid and electrolyte replacement therapy can increase the survival time of 15.4 Gy irradiated rats. The median survival time of rats irradiated with 15.4 Gy of 137-Cs gamma rays and receiving no treatment was 4.7 days with a 97% confidence interval of 4.6 to 4.8 days. The injection of 15 ml/day of LR (approximately equal to the fluid volume/day secreted via the bile in these rats) into the duodenum did not significantly increase median survival time. However, administering the same amount of LR to the peritoneal cavity significantly increased survival time ($p=.05$). This improvement in survival time was equivalent to that observed with BDL. Doubling the volume of LR injected into the peritoneal cavity resulted in a small further increase in median survival time. However, the addition of antibiotics to the LR injected into the abdomen produced the largest median survival time of 6.9 days, with some animals surviving as long as 9 days. This median survival time (6.9 days) approaches the median survival time of 7.9 days for animals receiving the same whole-body dose but with most of the GI tract exteriorized and protected by lead shielding during exposure. In animals surviving 7 days or longer after 15.4 Gy whole body or intestinal irradiation due to fluid and antibiotic treatment, there was significant regeneration of the mucosa as compared to untreated irradiated animals that succumbed 3 to 5 days after exposure. However, the lungs and kidneys of LR plus antibiotic treated animals dying more than 7 days post irradiation showed severe hemorrhaging, edema, and congestion at death. The incidence of diarrhea (>95%) before death was not affected by the various forms of fluid and electrolyte therapy.

The results of this experiment indicate that fluid and electrolyte loss, particularly via the bile, is the principal mechanism for acute intestinal radiation death in the rat. Evidence for this is the following:

1) Continuous drainage of bile in the unirradiated rat is lethal with a survival time comparable to untreated supralethally irradiated rats (Table 12). Fluid replacement with LR prevents this early death (Table 12). This indicates that the critical bile components necessary for survival are fluid and electrolytes.

2) Duodenal injection of fluid and electrolytes does not prolong survival of irradiated rats or irradiated bile drained rats, whereas injection of the same amount of fluid

TABLE 13

Effect of Fluid Replacement on Median Survival Time of 15.4 Gy Whole-Body Irradiated Rats

Treatment	No. of Rats	Median Survival Time[1,2]
Irradiated Control	38	4.6(4.4-4.9)
Irradiated with Fluid Replacement (15 cc/day Duodenal Injection)	8	4.2(3.9-4.6)
Irradiated with Fluid Replacement (15 cc/day Peritoneal Injection)	10	5.8(5.0-6.3)
Irradiated with Fluid Replacement (30 cc/day Peritoneal Injection)	10	6.0(4.4-6.6)
Irradiated with Fluid Replacement + Antibiotics (30 cc/day Peritoneal Injection)	10	6.9(6.0-8.2)
Irradiated and Bile Duct Ligated	22	6.1(5.7-6.8)
15.4 Gy Whole-Body Irradiated with Exteriorized Intestine Shielded	16	7.9(6.5-8.8)

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1. Time in Days after Irradiation.

2. Values in Parentheses are 95% or Greater Confidence Limits.

and electrolytes into the peritoneal cavity significantly increases survival time in these animals (Table 13). Fluid and electrolytes are poorly absorbed by the irradiated intestine and this is circumvented by injecting the fluid and electrolytes into the peritoneal cavity where they are readily available to the vascular system.

Some of the antibiotic and fluid treated animals survived as long as animals receiving the same radiation dose but which had their intestine shielded during irradiation (Table 13). Therefore, fluid replacement plus antibiotic treatment almost compensates for the decrease in survival time caused by radiation injury to the intestine. Nevertheless, all fluid and antibiotic treated 15.4 Gy irradiated animals eventually died. Lethality in this case may be due to malnutrition resulting from prolonged radiation-induced anorexia and other organ system failures, namely the lung, kidney, and hematopoietic system. Some of these organ system failures are probably treatment related (i.e., edema from fluid overloading and antibiotic toxicity) rather than caused by radiation alone. Therefore, a slow continuous IV infusion of colloid or crystalloid solutions to reduce lung and kidney damage combined with total parenteral feeding to correct for nutritional imbalance may be a better form of fluid therapy for intestinal radiation injury than the crude bolus injections used in the present study. Treatment for hematopoietic injury will of course also be needed to prevent acute mortality from these large radiation doses.

Changes in body fluid compartments in normal, BDL and antibiotic decontaminated animals after irradiation (Experiments 6 and 8 of Work Statement).

Conventional and antibiotic decontaminated rats, matched in body weight, were exposed to 0, 6, 12 and 18 Gy of gamma radiation. Some unirradiated animals were fasted during the course of the experiment to control for the absence of food intake by the irradiated animals. Some of the irradiated rats were subjected to bile duct ligation or choledochostomy one day after radiation exposure. At various times after irradiation, total body water was measured by isotopic dilution of tritiated water, extracellular fluid space (ECS) was measured by isotopic dilution of 51-Cr labelled EDTA, the plasma volume (PLV) by isotopic dilution of 125-I-albumin and the red blood cell volume (RBCV) by isotopic dilution of 51-Cr labelled RBC. The intracellular space (ICS) was calculated by the difference between total body water and the extracellular fluid space. The hematocrit and plasma DAO activity were also measured in these animals. In each rat the amount of radioactivity in the total intestinal tract was measured and this activity was subtracted from the activity injected. The purpose of this is to correct for fluid in the GI tract and possible non-equilibration of the isotopes in the GI tract fluid volume. It is believed that changes in fluid compartments after irradiation which are independent of changes in the GI tract reflect the more physiologically important parameters of radiation injury in these animals.

Tables 14 and 15 give the actual volumes of various body compartments as a function of time after irradiation, radiation dose and treatment (i.e., BDL, antibiotic decontamination or choledochostomy).

These data lead to the following conclusions:

1. At gamma ray doses (12 and 18 Gy) that produced acute GI death, the most sensitive fluid compartments are the plasma volume (PLV) and extracellular space (ECS). Decrease in these spaces lead to hemoconcentration as evidenced by an elevated hematocrit. BDL delayed the decrease in these two fluid compartments by 1 to 2 days, which is similar to the prolongation of survival observed in supralethally BDL irradiated rats (Figure 12).

2. The most important finding with regard to diamine oxidase measurement (an index of mucosal damage) is that changes in the levels of this enzyme correlate well with the changes in plasma volume and extracellular space of conventional and decontaminated animals following irradiation. This correlation supports the concept that radiation induced damage to the intestinal mucosa is the cause of the decrease in plasma volume, a decrease which is the principle terminal lethal mechanism of intestinal radiation death.

3. The results also confirm there is more than 1.5 mEq of sodium ion lost by the rat due to 18 Gy irradiation, an amount which is lethal (27). Approximately 85% of the body sodium is present in the extracellular fluid space (ECS). Therefore, the difference in the size of the ECS in an irradiated animal (near the time of death) as compared to the size of this space in a fasted unirradiated animal at the same time, multiplied by the sodium ion concentration in ECS gives a good estimate of the sodium ion lost due to irradiation. The sodium ion concentration in the extracellular fluid space (0.14 mEq/ml) does not appear to change as a result of irradiation (28,29), which is expected when the fluid lost as diarrhea is isotonic (28,29,40). Calculation of net sodium loss is as follows:

$$Na^{+}(\text{loss}) = (\text{ECS}_{\text{0 Gy-fasted}} - \text{ECS}_{\text{18Gy}})\ (0.14\ \text{mEq/ml}) =$$

$$(46.2\ \text{ml} - 32.1\ \text{ml})\ (0.14\ \text{mEq/ml}) = 1.96\ \text{mEq}$$

Thus, by a different method than used previously (27) we have confirmed the substantial loss of sodium in supralethally irradiated animals. This is in contradiction to other reports of only minimal loss of sodium in supralethally irradiated rats (43,44).

TABLE 14

Time (day)	Body WT. (gm)	Total Body water (ml)	ICS (ml)	ECS (ml)	Vas. vol. (ml)	Plasma vol. (ml)	RBC vol. (ml)	Hct.	DAO (u/PLV)
0 Gy									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	2.822±0.314
3	199.5±3.4	147.7±5.0	96.3±5.1	51.4±2.2	18.4±0.9	10.4±0.39	----------	0.45±0.01	2.721±0.404
6	217.2±3.5	149.4±2.9	93.9±1.8	55.5±1.6	19.9±0.4	11.0±0.07	----------	0.45±0.01	2.763±0.388
9	236.3±2.6	164.1±1.7	109.2±3.5	54.9±0.7	20.3±1.2	11.7±0.44	----------	0.44±0.01	3.255±1.479
12	240.4±4.1	159.1±2.7	110.0±2.6	57.7±0.4	22.9±0.9	12.9±0.60	----------	0.44±0.01	3.322±0.494
0 Gy Fasted									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	2.822±0.314
1	184.1±4.0	138.7±2.1	87.1±1.7	51.6±0.4	---------	----------	----------	----------	-----------
2	168.3±4.9	136.2±2.3	85.7±1.7	50.5±1.2	14.1±0.2	6.90±0.11	4.70±0.10	0.42±0.01	1.896±0.309
3	151.7±5.5	128.3±3.8	83.1±1.0	45.3±2.9	14.3±0.2	6.98±0.07	4.51±0.07	0.49±0.01	1.368±0.420
4	139.3±6.8	127.6±5.0	80.5±4.6	47.0±0.2	14.4±0.3	6.97±0.16	4.58±0.10	0.51±0.01	0.700±0.171
5	135.7±2.7	116.0±2.4	71.9±1.5	44.1±1.0	12.7±0.3	5.57±0.17	4.58±0.11	0.53±0.01	0.663±0.302
6 Gy Conventional Rats									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	2.822±0.314
2	168.3±3.3	139.9±3.2	87.9±1.8	52.0±1.4	15.7±0.6	9.08±0.46	4.12±0.10	0.41±0.01	0.527±0.092
3	176.7±4.1	139.8±3.6	87.3±2.3	52.5±3.0	16.4±0.3	9.57±0.24	4.52±0.06	0.41±0.01	0.436±0.127
4	180.8±4.0	135.9±7.0	81.4±4.6	54.1±2.5	16.8±0.4	10.3±0.12	4.41±0.21	0.40±0.01	1.176±0.303
6	192.2±1.4	153.9±0.8	98.3±1.4	55.7±1.0	16.4±0.6	10.6±0.48	3.94±0.10	0.38±0.01	1.221±0.091
9	208.8±2.7	169.9±5.3	108.0±3.4	61.9±3.1	16.8±0.4	11.0±0.40	4.07±0.17	0.35±0.01	1.270±0.124
12	228.0±5.2	179.6±7.5	113.2±4.8	66.5±2.9	17.5±0.8	12.7±1.17	3.42±0.27	0.31±0.02	1.289±0.249
12 Gy Conventional Rats									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	2.822±0.314
2	168.2±2.8	132.4±0.4	80.3±1.3	52.1±1.1	16.1±0.4	9.21±0.28	4.42±0.15	0.42±0.02	1.033±0.202
3	158.1±1.9	125.4±4.9	78.8±2.9	46.6±4.5	14.2±0.4	7.22±0.23	4.60±0.13	0.48±0.01	0.183±0.030
4	133.9±2.5	99.6±4.8	61.7±4.1	37.9±0.7	12.8±0.5	6.00±0.32	4.09±0.10	0.53±0.01	0.017±0.003
5	134.2±3.9	110.3±6.3	68.9±3.0	41.4±3.7	13.6±0.5	7.07±0.34	3.94±0.15	0.46±0.02	0.141±0.047
6	125.6±3.7	98.0±5.7	59.8±2.3	38.2±3.4	13.9±0.3	7.92±0.42	3.69±0.17	0.43±0.01	0.125±0.014
7	123.0±4.4	89.0±5.7	55.1±2.5	33.9±3.2	14.5±0.5	8.22±0.33	4.01±0.26	0.42±0.03	0.365±0.067
8	120.2±4.6	99.7±4.8	62.9±4.1	40.1±1.9	13.7±0.9	8.87±0.62	2.96±0.30	0.30±0.02	0.472±0.147
9	141.1±11	114.0±7.4	70.8±4.0	43.1±3.7	---------	----------	----------	0.15±0.02	----------
18 Gy Conventional Rats									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	2.882±0.314
1	174.5±2.6	133.4±3.2	80.6±5.1	50.3±0.9	15.2±0.7	8.74±0.48	4.36±0.20	0.42±0.01	2.006±0.316
2	167.3±3.5	129.0±3.9	79.2±2.8	49.7±1.2	14.9±0.5	8.07±0.35	4.20±0.07	0.44±0.01	1.388±0.144
3	148.8±2.8	118.0±2.5	74.9±1.8	38.8±1.4	14.3±0.3	6.78±0.10	4.21±0.08	0.51±0.01	0.102±0.011
3.5	134.6±1.4	103.8±3.6	72.5±3.3	32.1±0.9	13.3±0.1	5.55±0.18	3.82±0.09	0.58±0.01	0.020±0.006

TABLE 15

Time (day)	Body WT. (gm)	Total Body water (ml)	ICS (ml)	ECS (ml)	Vas. vol. (ml)	Plasma vol. (ml)	RBC vol. (ml)	Hct.	DAO (u/PLV)
18 Gy Bile Duct Ligated Rats									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	2.822±0.314
2	159.9±4.7	126.4±2.2	77.0±3.1	49.4±1.0	14.6±0.3	8.72±0.24	3.94±0.14	0.40±0.01	0.710±0.108
3	150.4±3.6	119.1±2.8	77.1±2.6	42.0±0.8	14.8±0.6	8.88±0.16	3.77±0.33	0.40±0.02	0.146±0.033
4	136.9±3.4	109.9±2.4	71.7±1.7	38.2±1.5	14.3±0.5	8.13±0.62	3.99±0.07	0.43±0.01	0.133±0.014
5	125.9±4.6	95.3±3.1	64.5±1.5	30.8±1.7	10.8±0.3	5.65±0.13	3.31±0.23	0.48±0.01	0.228±0.062
0 Gy Fasted-Bile Duct Cannulated									
0	187.9±2.1	137.5±2.1	85.9±1.3	53.8±0.6	16.0±0.4	9.38±0.26	4.41±0.12	0.42±0.01	-----------
1	172.2±3.2	127.4±2.9	85.1±2.8	42.4±1.4	--------	---------	---------	0.43±0.02	-----------
2	155.8±6.7	121.8±6.0	81.3±3.3	40.5±3.0	--------	---------	---------	0.48±0.01	-----------
3	146.0±6.6	111.9±6.3	77.9±3.5	34.0±2.8	--------	---------	---------	0.49±0.02	-----------
6 Gy Decontaminated Rats									
0	156.3±2.8	111.2±4.8	70.8±2.1	41.9±1.9	14.8±0.4	8.92±0.30	4.14±0.14	0.39±0.01	1.837±0.228
2	143.4±2.8	108.5±2.7	65.5±2.4	43.0±0.6	14.2±0.4	8.35±0.28	3.66±0.07	0.41±0.01	1.079±0.374
3	136.0±5.8	107.5±3.5	67.1±2.2	40.5±1.6	12.7±0.7	7.32±0.69	3.72±0.14	0.43±0.01	0.523±0.147
4	142.5±4.7	113.0±2.8	73.6±1.4	39.3±1.5	13.3±0.7	7.59±0.65	3.76±0.09	0.41±0.02	1.133±0.286
6	159.4±5.3	114.6±4.2	71.9±3.2	42.6±1.1	14.6±0.6	9.34±0.36	3.68±0.13	0.37±0.01	0.976±0.261
9	176.4±5.6	128.6±4.1	80.8±2.5	47.8±1.7	13.4±0.6	9.51±0.65	3.34±0.24	0.29±0.02	0.969±0.132
12	194.7±6.9	134.9±7.6	83.1±4.4	51.8±3.3	13.8±0.4	10.2±0.28	2.88±0.16	0.28±0.01	0.997±0.204
12 Gy Decontaminated Rats									
0	156.3±2.8	111.2±4.8	70.8±2.1	41.9±1.9	14.8±0.4	8.92±0.30	4.14±0.14	0.39±0.01	1.837±0.228
2	132.7±3.3	99.6±1.9	63.1±1.2	36.5±1.2	13.5±0.6	8.27±0.49	3.46±0.09	0.42±0.02	2.139±0.607
3	133.1±4.0	96.4±3.2	61.9±2.2	34.5±1.2	13.1±0.4	7.06±0.21	3.97±0.25	0.47±0.01	0.278±0.097
4	110.5±2.4	85.9±2.2	56.9±1.9	29.0±0.9	11.3±0.3	5.57±0.20	3.50±0.09	0.52±0.01	0.142±0.061
5	116.6±5.2	83.6±7.4	51.6±3.9	32.0±3.6	12.1±0.4	6.50±0.39	3.56±0.09	0.46±0.02	0.667±0.335
6	114.5±4.2	94.1±4.4	58.0±4.2	36.1±0.5	12.4±0.6	7.29±0.56	3.34±0.12	0.44±0.02	0.470±0.123
7	120.0±5.1	96.6±1.8	57.7±1.2	38.9±1.3	11.1±0.3	7.36±0.33	3.16±0.11	0.42±0.01	0.391±0.155
8	128.8±4.8	98.7±2.9	56.5±1.4	42.3±1.6	13.6±0.8	8.62±0.50	3.05±0.21	0.33±0.02	0.745±0.295
9	138.9±5.0	107.8±3.8	68.4±1.3	39.4±3.0	13.2±0.5	10.0±0.35	2.58±0.29	0.27±0.02	0.995±0.293
10	135.7±3.7	113.8±5.9	71.9±6.5	41.9±1.5	11.5±0.4	9.77±0.49	1.54±0.14	0.14±0.03	0.387±0.052
18 Gy Decontaminated Rats									
0	156.3±2.8	111.2±4.8	70.8±2.1	41.9±1.9	14.8±0.4	8.92±0.30	4.14±0.14	0.39±0.01	1.837±0.228
2	128.7±4.6	101.0±0.6	63.2±0.4	37.9±1.0	13.5±0.2	7.76±0.35	3.56±0.06	0.42±0.02	1.024±0.306
3	117.7±4.8	88.2±5.0	56.5±3.5	31.7±1.6	12.1±0.5	6.26±0.35	3.81±0.13	0.50±0.01	0.311±0.234
3.5	111.2±2.0	82.4±2.3	53.9±1.3	28.5±1.0	10.9±0.5	5.21±0.15	3.87±0.16	0.52±0.01	0.073±0.040
4	111.3±2.5	77.5±3.9	50.9±2.1	26.6±2.0	10.3±0.1	4.69±0.12	3.49±0.04	0.57±0.02	0.021±0.007

Finally, opponents of the fluid and electrolyte loss theory of intestinal radiation death have argued that substantially more sodium loss by unirradiated bile cannulated rats will not cause death (44). We have proven this to be incorrect. Cannulation of the bile duct of unirradiated rats with bile drainage to the outside of the animal is lethal unless accompanied by fluid and electrolyte replacement (Table 12). Furthermore, the magnitude of sodium loss in unirradiated bile drained, fasted rats, just prior to death, is similar to sodium loss by supralethally irradiated rats as the following calculation shows:

Sodium loss by unirradiated, bile drained rats =

$(\mathrm{ECS}_{\text{0Gy-fasted}} - \mathrm{ECS}_{\text{0Gy-fasted, bile drainage}})\ (0.14) =$

$(45.3\ \mathrm{ml} - 34.\ \mathrm{ml}) = 1.58\ \mathrm{mEq}$

This value of 1.58 mEq is to be compared with the 1.5 to 2.0 mEq of sodium lost by rats dying of intestinal radiation injury. This is a lethal loss of sodium.

Experiment with 1.2 MeV fission neutrons at AFRRI (Supplemental Contract P00003 of Work Statement).

As part of this contract Dr. Joseph Geraci traveled to the Armed Forces Radiobiology Research Institute (AFRRI) to conduct additional contract experiments over a period of 6 months using the AFRRI TRIGA Reactor and 60-Co radiation source. For this purpose a small laboratory and animal holding facility were set up in order to duplicate some of the experiments conducted previously at the University of Washington.

The purpose of the first experiment conducted at AFRRI was to correlate the results of 60-Co gamma irradiation of rats at AFRRI with similar results obtained with the University of Washington 137-Cs gamma ray source. Rats were bilaterally exposed with a range of 60-Co gamma doses and were followed to measure survival time and percent survival at 5 days and 30 days post exposure. Data obtained for median survival time as a function of dose (Figure 18) showed there were no significant differences in the results produced by irradiation at AFRRI as compared to irradiation at the University of Washington. This indicates that the handling of animals and the radiation dosimetry were conducted in a comparable manner at the two sites. Animals were then irradiated with AFRRI fission neutrons to determine survival time and percent survival as a function of radiation dose. These data have been compared with similar experiments conducted at the University of Washington using 8 MeV cyclotron-produced neutrons and 137-Cs gamma rays (Figure 19). The results show a biphasic response curve with fission neutrons similar to that previously observed for the other radiations at the University of Washington. Median survival time with fission neutrons was the same as measured with 8 MeV neutrons or 137-Cs gamma rays for radiation doses which produced pure intestinal

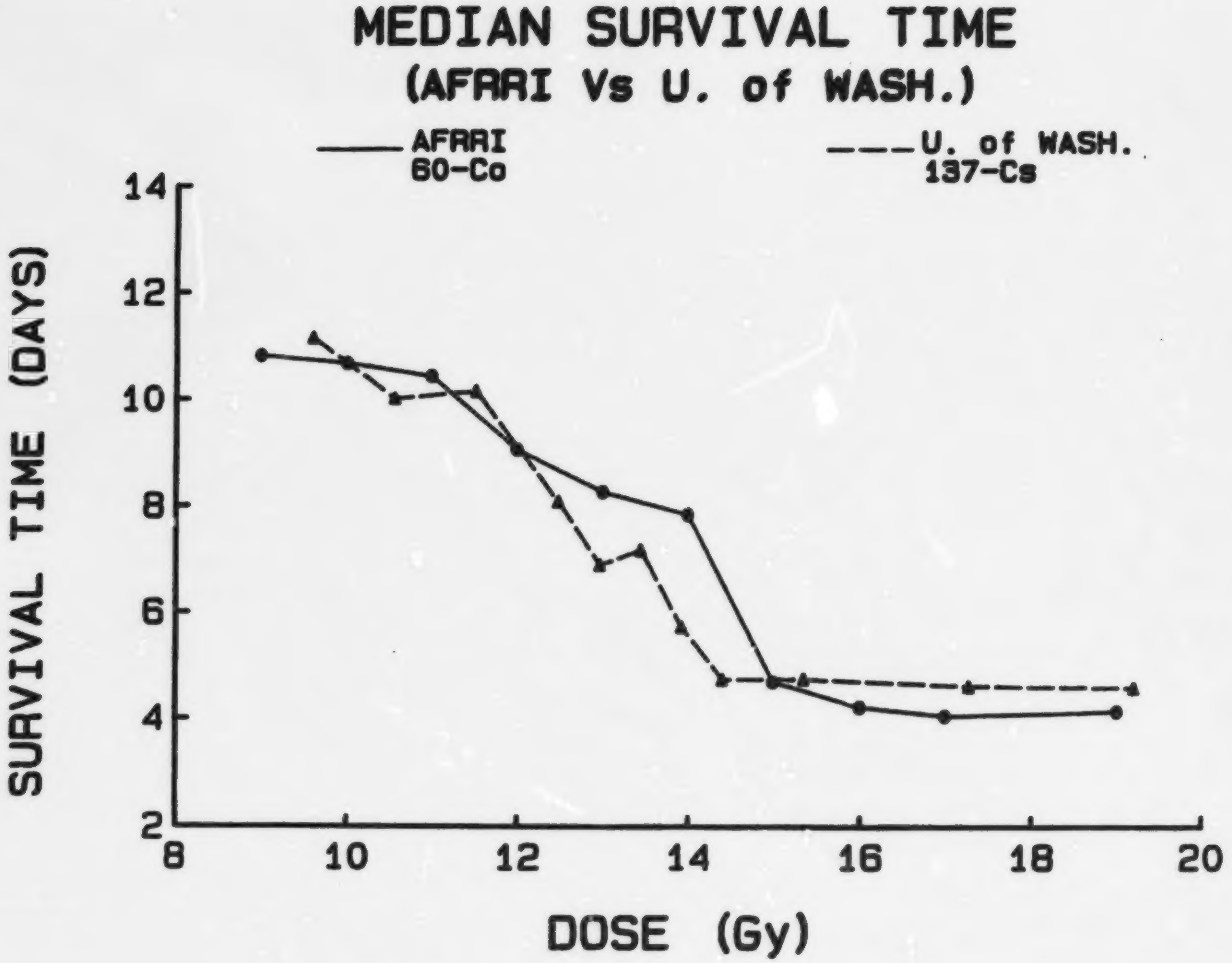

Figure 18. Dose survival time curves for 60-Co gamma irradiated animals at AFRRI and 137-Cs gamma irradiated animals at the University of Washington. Each point represents the median survival time of 8 to 40 rats after exposure to the indicated dose.

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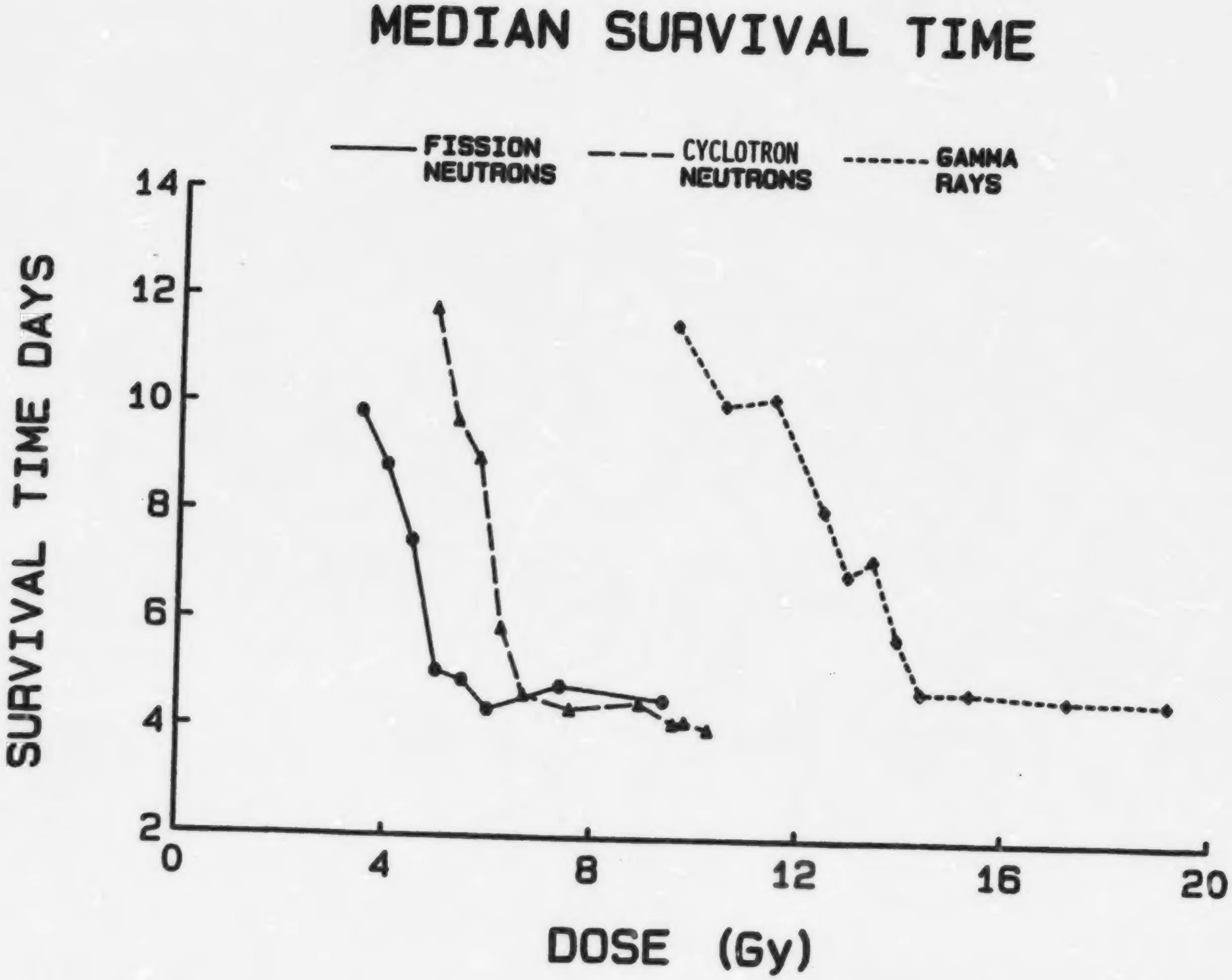

Figure 19. Survival time of fission neutron, cyclotron neutron, and 137-Cs gamma irradiated animals as a function of dose. Each point represents the median survival time of 8 to 40 animals.

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death (i.e., the dose range where survival time becomes constant with increasing radiation dose). However, the threshold dose for pure intestinal death was lower with fission neutrons than with 8 MeV neutrons. The effect of antibiotic decontamination of the intestinal tracts of animals exposed to fission neutrons is shown in Figure 20. Similar to previous results at the University of Washington, decontamination (see Figure 8) had no effect on survival time at radiation doses (6-10 Gy) which produced pure intestinal death. However, decontamination did increase survival time by 1 to 5 days in the dose range (4.0, 6.0 Gy) where death is caused by a combination of hematopoietic and intestinal injuries, which is similar to the results obtained with 137-Cs gamma rays and 8 MeV neutrons at the University of Washington.

The influence of bile duct ligation on fission neutron irradiated conventional and decontaminated rats also was studied (Figure 21). In agreement with previous work at the University of Washington with gamma rays and neutrons (see Figure 12), bile duct ligation of fission neutron irradiated rats resulted in an increase in survival time in the radiation dose range which produced pure intestinal death.

In Figure 22 is shown the dose-percent mortality curves for intestinal radiation death (5 day survival) and hematopoietic death (30 day survival) produced by fission neutrons, 8 MeV neutrons, and 137-Cs gamma rays. These data show that fission neutrons are more effective in producing both types of death as compared to 8 MeV cyclotron neutrons or gamma rays. Table 16 gives the actual LD50/30 day and LD 50/5 day values for each radiation. In comparison to the value for intestinal death, the RBE value for fission neutrons for hematopoietic death were significantly higher than the RBE values for the 8 MeV neutron beam. This effect probably is due to a change in the biological quality of the fission neutron beam as a function of tissue depth because of the marked attenuation of the low energy neutrons in this beam. The hematopoietic system is located nearer the surface of the animal than is the intestinal tract. If this interpretation is correct, the difference in RBE values for hematopoietic versus intestinal death would be even greater in larger animals, such as dogs or humans.

Information concerning the percent of animals developing diarrhea as a function of radiation dose and the median time for onset of diarrhea as a function of dose for animals irradiated at AFRRI with fission neutrons is shown in Figure 23 and Table 17. These results also show the effects of antibiotic decontamination of the GI tract and bile duct ligation on incidence and time of diarrhea. In agreement with neutron studies carried out at the University of Washington, bile duct ligation prolonged the time for onset of diarrhea and markedly increased the threshold dose required to produce diarrhea.

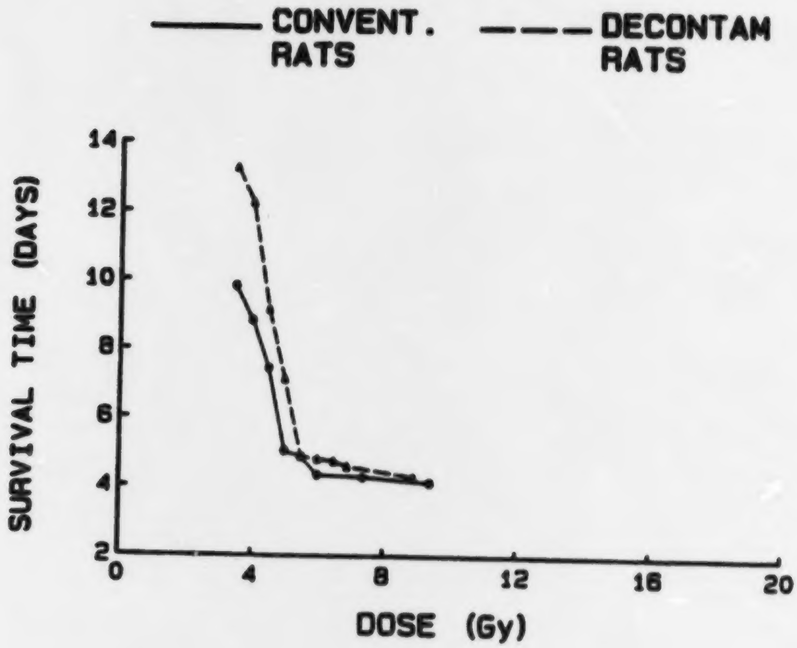

Figure 20. Survival time of untreated, GI decontaminated animals after exposure to various doses of fission neutrons. Each point represents the median survival time of 8 to 16 animals.

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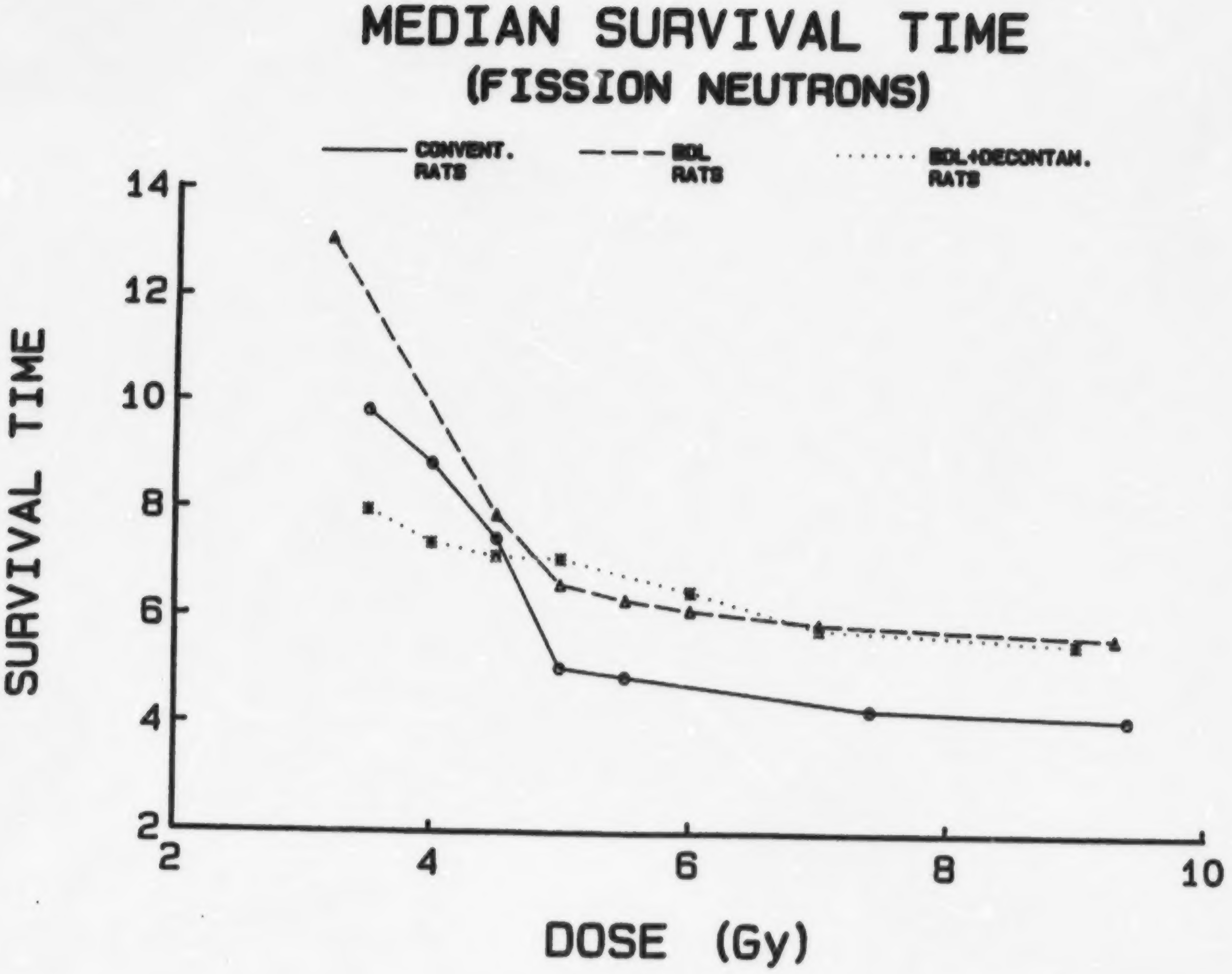

Figure 21. The effect of BDL on survival time of conventional and GI decontaminated rats after exposure to graded doses of fission neutrons. Each point represents the median survival time of at least 8 animals. Error bars are omitted for clarity. 95% confidence limits were 2 to 10% of the mean values.

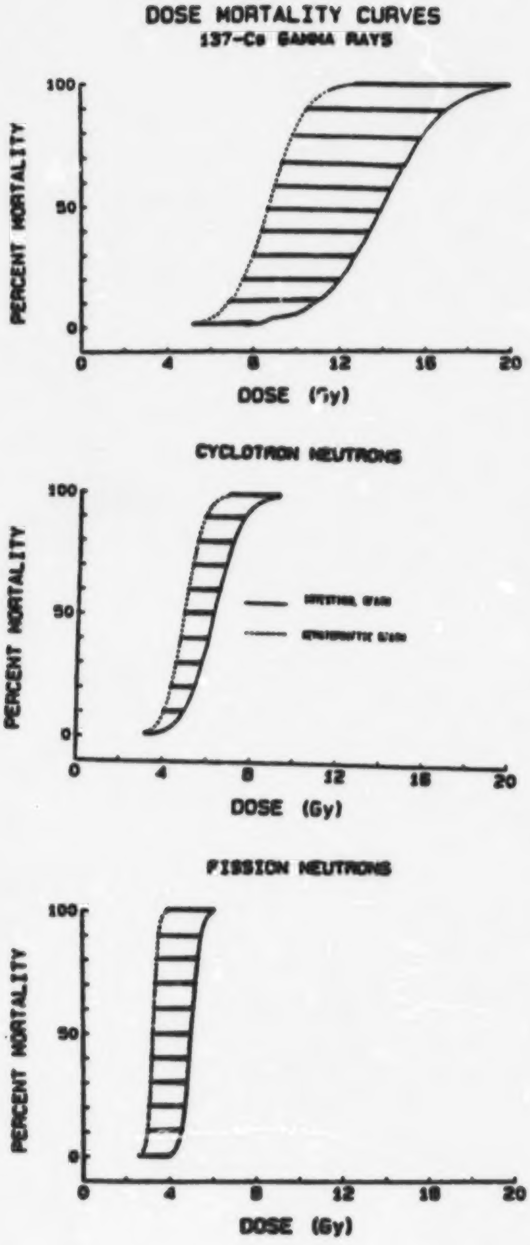

Figure 22. Dose mortality curves resulting from intestinal and hematopoietic injury after exposures to 137-Cs gamma rays, cyclotron neutrons, or fission neutrons.

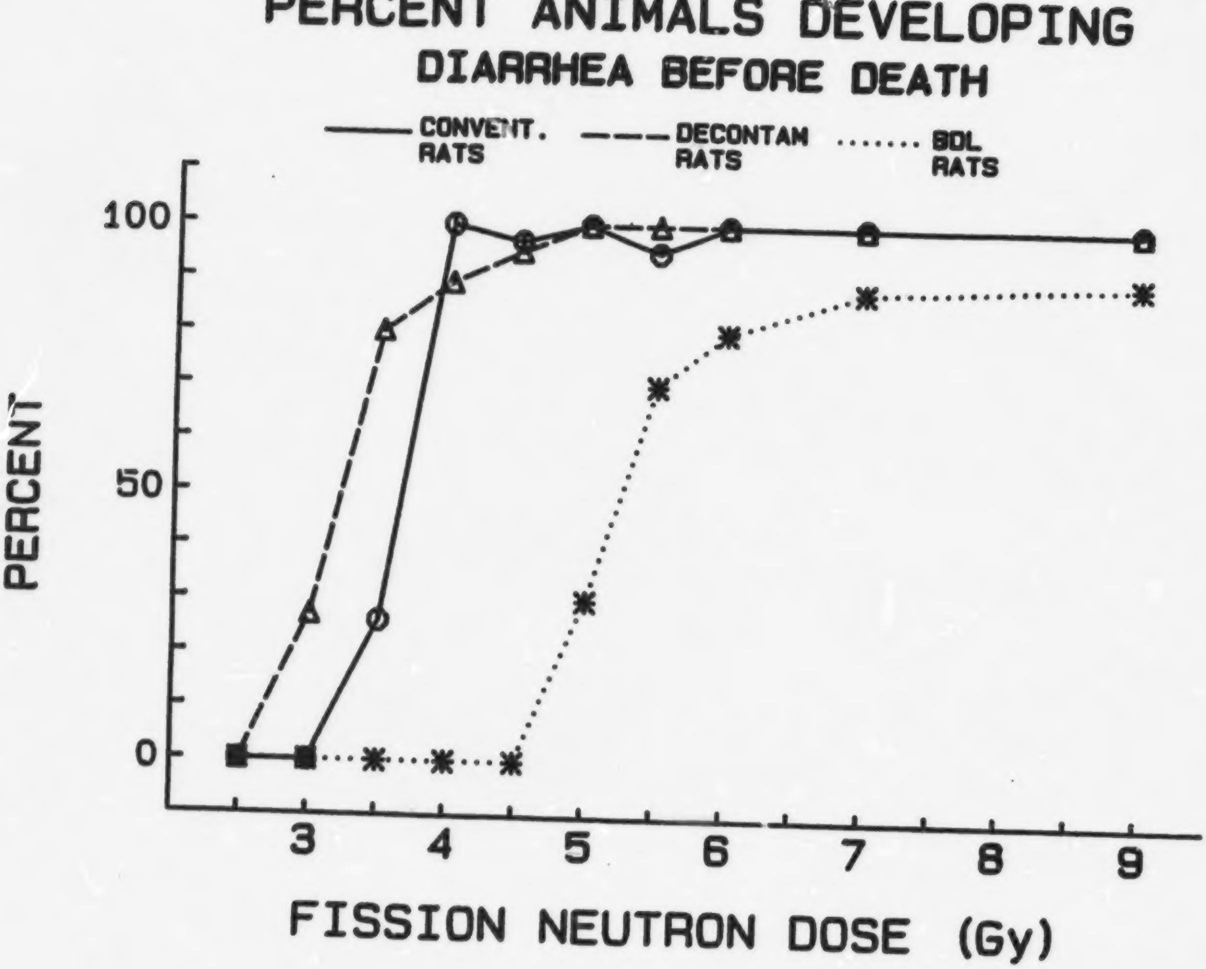

Figure 23. Dose response curves for the development of diarrhea in conventional, GI decontaminated, or BDL rats after fission neutron exposure. Each point represents the percentage of 8-24 animals that developed diarrhea at the indicated dose.

TABLE 16

LD 50/30 and LD 50/5 day for Fission Neutron, Cyclotron Neutron, and 137-Cs Gamma-Irradiated Rats

Radiation	LD 50/30 day (Gy)	RBE	LD 50/5 day (Gy)	RBE
137-Cs γ rays	8.75 (8.04-9.50)	-	14.1(13.4-14.7)	-
C-Neutrons	4.90 (4.56-5.27)	1.8	6.25 (6.10-6.40)	2.3
F-Neutrons	3.25 (3.08-3.43)	2.7	5.00 (4.72-5.30)	2.8

C - Neutrons = 8 MeV mean energy cyclotron produce neutrons;
F - Neutrons = fission neutrons

TABLE 17

Median Time (Days) for Diarrhea Onset
After Fission Neutron Irradiation

Dose (GY)	Conventional Rats	GI Decontaminated Rats	BDL Rats
2.5	*	*	*
3.0 - 3.2	*	*	*
3.5	*	3.98	*
4.0	4.16	3.85	*
4.5	3.84	3.58	*
5.0	3.91	3.51	*
5.5	3.65	3.45	5.08[1]
6.0	3.31	3.30	5.02[1]
6.5	ND	3.46	ND
6.9 - 7.4	3.22	3.05	4.36[1]
8.9 - 9.4	3.22	2.86	4.25[1]

---

* Less than 50% of animals develop diarrhea before death from radiation or sacrificed at 30 days

[1] Times are significantly ($P<.05$) longer than conventional or decontaminated rats.

ND = Not Done

Studies at AFRRI also were carried out to determine the effectiveness of fluid and electrolyte replacement therapy in rats receiving lethal doses of fission neutrons (Figure 24). These results show that injection of lactate-Ringer solution directly into the abdominal cavity duplicates the increase in survival time produced by bile duct ligation in supralethally, fission neutron irradiated rats. This is additional evidence that the mechanism by which bile duct ligation increases survival time in the intestinal radiation death dose range is the slowing down of the rate of fluid and electrolyte loss.

SECTION 4

DISCUSSION AND CONCLUSIONS

The possibility of exposure to neutrons or to large doses of gamma radiation from nuclear devices and the use of radioprotectors accompanied by improvements in techniques to treat hematopoietic injury have increased the importance of intestinal radiation damage in the medical management of radiation casualties. The reason is that exposure to neutrons or prior treatment with radioprotectors significantly reduce the dose differential needed to produce intestinal and hematopoietic injuries as illustrated in Figure 25 for rats. As a consequence, the dose range (indicated by the cross hatched areas) within which the best available medical treatment (bone marrow transplantation) is effective in preventing lethality is significantly smaller after neutron exposure and/or pretreatment with radioprotectors (as compared to gamma irradiation alone).

The ineffectiveness of bone marrow transplantation after neutron exposure as compared to its effectiveness after gamma exposure of dogs (83), primates (84) and mice (85) clearly demonstrates the significance of this reduced dose window for hematopoietic treatment, which is due to an increase in the contribution of intestinal injury to lethality. Hence, successful medical treatment of most individuals who have taken radioprotectors before irradiation and/or who have been exposed to neutrons or high doses of gamma rays will require optimum treatment of the injury to both the hematopoietic and intestinal systems. The importance of management of intestinal radiation injury has recently been dramatically demonstrated by the fact that approximately 10 Chernobyl victims receiving bone marrow transplantation succumbed within three weeks after the accident, most likely from lethal intestinal injury.

Unfortunately, whereas the physiological mechanisms of hematopoietic injury are reasonably understood and its treatment is reasonably well worked out, this has not been true for intestinal radiation injury. There is general agreement, that intestinal radiation death is the result of denudation of the intestinal mucosa after supralethal whole body irradiation. What has been uncertain is the nature of the physiological derangements that lead to death following mucosal injury. Some

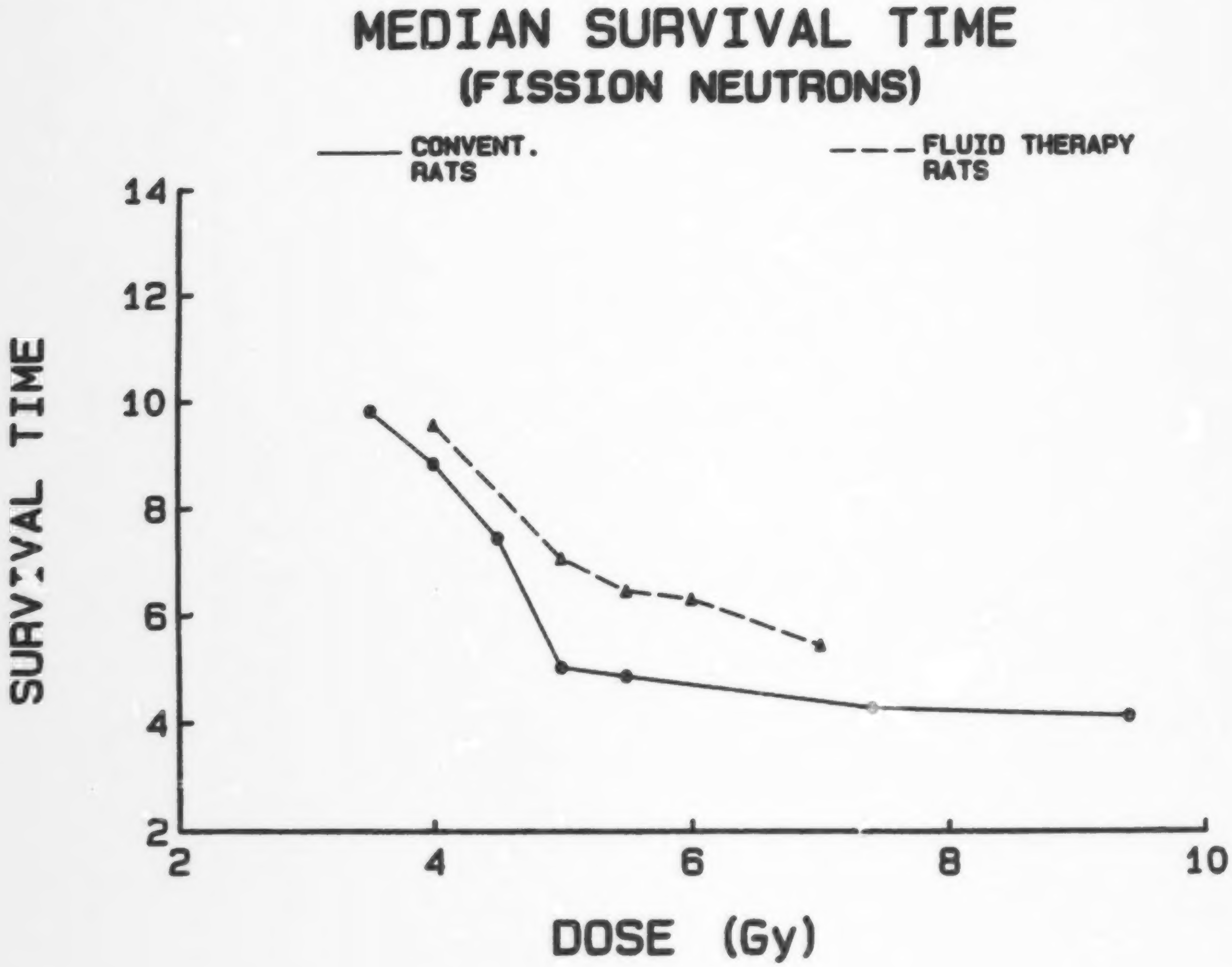

Figure 24. Survival time of animals receiving fluid replacement therapy via the peritoneal cavity as a function of fission neutron dose. Each point represents the median survival time of 10 to 16 animals.

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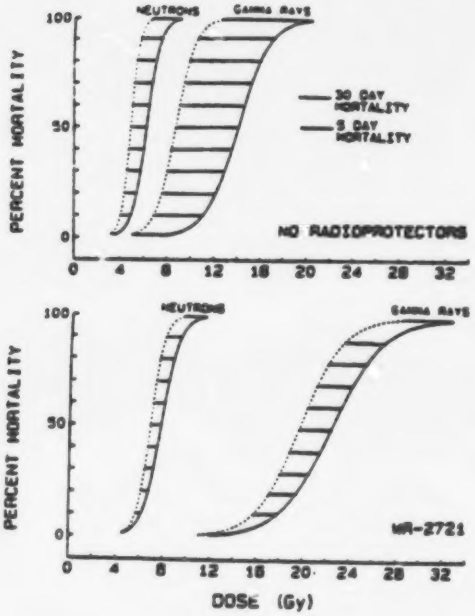

Figure 25. Dose range for which currently available medical treatment may be effective in preventing death of rats exposed to cyclotron neutrons or 137-Cs gamma rays, with and without prior treatment with WR-2721. The curves represent logistic functions which are based on probit curves that fit 7 to 12 data points (8 to 40 animals per point) with a Chi-square greater that 95%. The data points have been omitted from the graph for clarity. The cross-hatched areas indicate the dose regions where best available medical procedures (e.g., bone marrow transplantation) can prevent death in these rats. The assumption is that there currently exist no medical procedures to prevent GI death.

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investigators (86-89) have postulated that the mechanism of intestinal radiation death is partially due to the development of sepsis and endotoxemia resulting from escape into the body of bacteria and bacterial endotoxin from the damaged intestine. Another mechanism frequently cited as primary involves fluid and electrolyte loss via diarrhea. This in turn causes a decrease in the exchangeable sodium space, interstitial fluid space, and plasma volume, causing hypovolemic shock and death. Some investigators (27-29,40) suggest this loss of fluid and electrolytes before death is a direct result of the inability of the mucosa depleted gut to effectively transport fluid and electrolytes out of the intestinal lumen. Alternatively, it has been proposed that fluid and electrolyte loss may be a consequence of an increase in the concentration of bile salts in the intestinal lumen after irradiation (30-33). Bile salts, which are mucolytic agents, increase in the intestinal tract because there is defective reabsorption of bile (32). The resulting mucus depleting action of bile salts on the irradiated intestinal epithelium may then alter transport of water and electrolytes and contribute to diarrhea (30,33), thus causing dehydration and death.

In the present contract research we have investigated all three of these possible mechanisms using a rat model and military significant endpoints. The major findings and their implications with respect to possible medical treatment are summarized below.

Endogenous enteric bacteria were found not to play a significant role in pure intestinal radiation death. These results suggest that, for radiation casualties without other trauma, it is not necessary and probably wise not to start systemic antibiotic therapy for some time after irradiation, i.e., not until hematopoietic injury begins to be expressed. The reason is prolonged use of high doses of antibiotics can lead to toxic effects to critical organs such as the kidney, liver and lung, as well as inhibition of recovery of the mucosa and bone marrow. It is known from radiation therapy experience in humans that antibiotics administered after irradiation can enhance radiation injury in normal tissues. Enhanced injury and/or delayed recovery have also been observed in our laboratory when animals were supralethally irradiated (17.3 Gy) with one femur shielded to protect against hematopoietic death, followed by fluid therapy and systemic administration of antibiotics (see Figure 26). However, the injection of non-absorbable antibiotics into the GI tract immediately after irradiation, to selectively remove potential pathogenic aerobic bacteria prior to the onset of significant hematopoietic injury, may reduce the incidence and severity of sepsis in radiation casualties with minimal effects on critical organs. In addition, selective decontamination of the GI tract may also reduce the amounts of systemic antibiotics needed to control infections. Further studies are necessary to determine if this will be the case.

Bile acids, per se, were shown to play little role in intestinal radiation death. Evidence for this is that

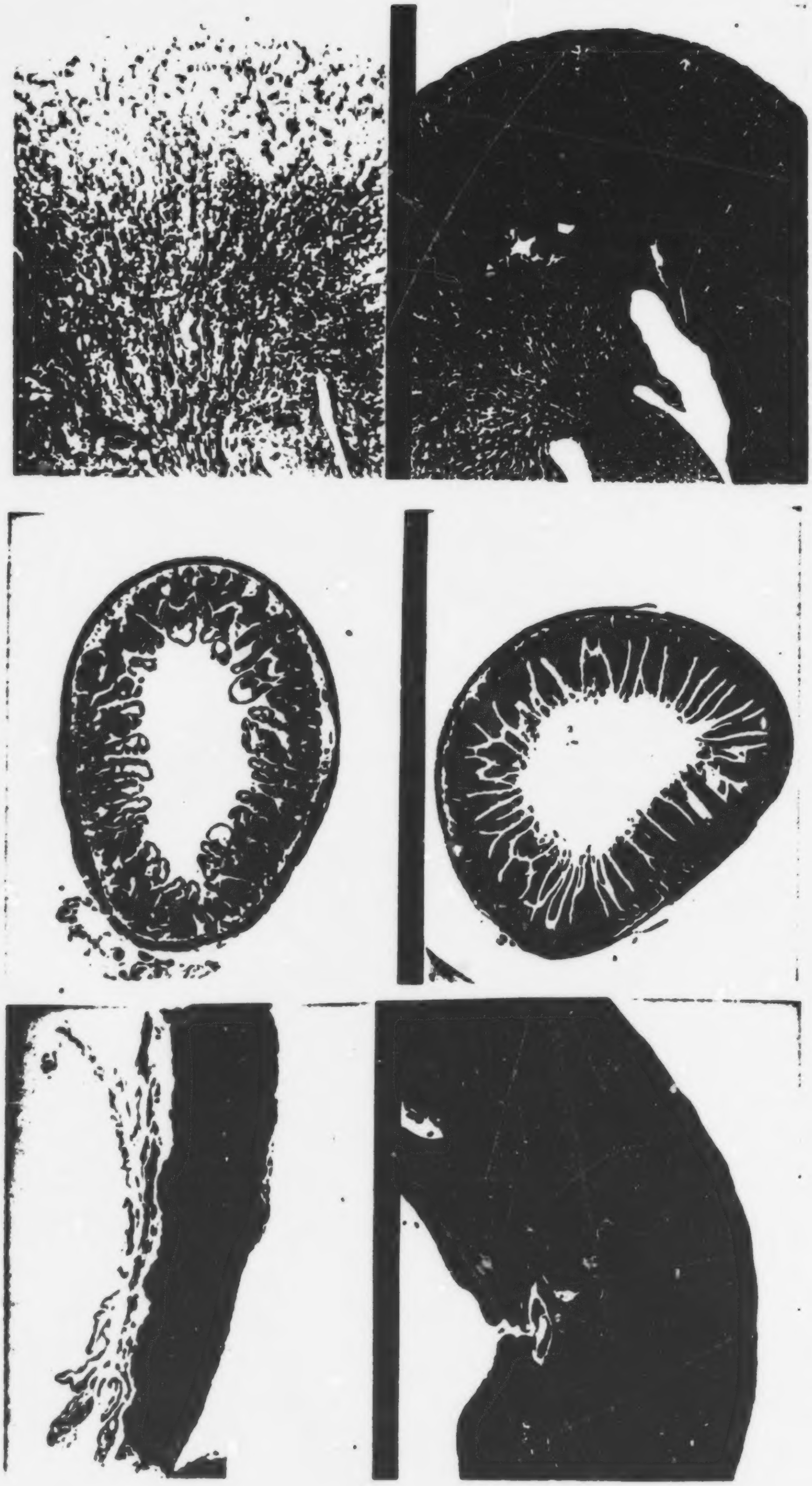

Fig. 26. Histology of Kidney (top), intestine (middle), and spleen (bottom), 10-11 days after 17.3 Gy gamma irradiation in animals receiving fluid therapy plus high dose systemic antibiotics (left) or fluid therapy alone (right). For these studies one femur was shielded to prevent lethality from hematopoietic injury. Marked dissolution of the cortex of the kidney and tubular abnormalities were observed in animals receiving antibiotics. Although there is substantial intestinal mucosal regeneration at these high doses, the mucosa of the antibiotic treated animals were more abnormal in that there were less villi, the villi were blunted and contained fewer epithelial cells. The spleens from antibiotic treated animals were also much smaller, with few, if any, regenerative foci as compared to the spleens from animals receiving fluid therapy alone. This clearly demonstrates that antibiotics can inhibit regeneration of hematopoietic system after irradiation.

cannulating the bile duct or exteriorizing bile flow did not increase survival time, even though these procedures protect all of the small intestine from the action of endogenous bile acids (18). However, it was also shown that preventing bile acids from entering the GI tract (by bile duct ligation) resulted in less severe injury to the intestinal mucosa, as measured by plasma DAO activity (see Figure 27). Therefore, the injection of the non-toxic cholestyramine resin into the GI tract immediately after irradiation may be an effective adjunct to fluid and electrolyte therapy. There is also indication that this binding of bile acids may promote the repair of the mucosa after irradiation, especially if combined with removal of gut bacteria with antibiotics (90). This needs to be further investigated using the rat model system.

The inability of the denuded mucosa of the irradiated small intestine to absorb fluid and electrolytes, resulting in hypovolemic shock, was found to be the major mechanism for intestinal death. This conclusion is based on the observation that the breakdown and recovery of the intestinal mucosa, as measured by plasma DAO activity is directly correlated with changes in plasma volume (PLV) and extracellular fluid space (ECS) in lethally irradiated animals (see Tables 14 and 15). This is further substantiated by the finding that injection of fluid and electrolytes into the intestinal lumen had no effect on survival time, whereas, injection of the same amount of fluid into the peritoneal cavity (where it is readily absorbed by the vascular system) significantly prolonged survival of supralethally whole body irradiated animals (Table 13). This increase in survival time was sufficient in some animals, who also received supplemental antibiotic treatment, for mucosal regeneration to occur resulting in prevention of intestinal death. These animals died from hematopoietic injury and/or lung and kidney damage, which may have been due to fluid overloading and antibiotic toxicity. Thus, there is the possibility that appropriate treatment of both hematopoietic and intestinal injury can result in recovery from relatively large neutron or gamma exposures.

Consequently, ways to optimize fluid and electrolyte replacement in the treatment of intestinal radiation injury need to be investigated. This should include studies on: (a) the effectiveness of isotonic versus hypertonic solutions administered during the critical phase of fluid and electrolyte loss, (b) the effectiveness of colloidal replacement therapy versus crystalloid therapy since crystalloids may cause more damage to the lung and kidney as compared to colloidal solutions, (c) evaluation of the effectiveness of sodium loading prior to the onset of intestinal radiation injury by administration of hypertonic sodium solutions with and without aldosterone to expand the extracellular fluid space and the exchangeable sodium space (aldosterone, a steroid hormone of the adrenal cortex, increases reabsorption of sodium by the renal tubules causing expansion of the extracellular fluid space (91)), (d) to evaluate the effectiveness of total parenteral nutrition to correct for

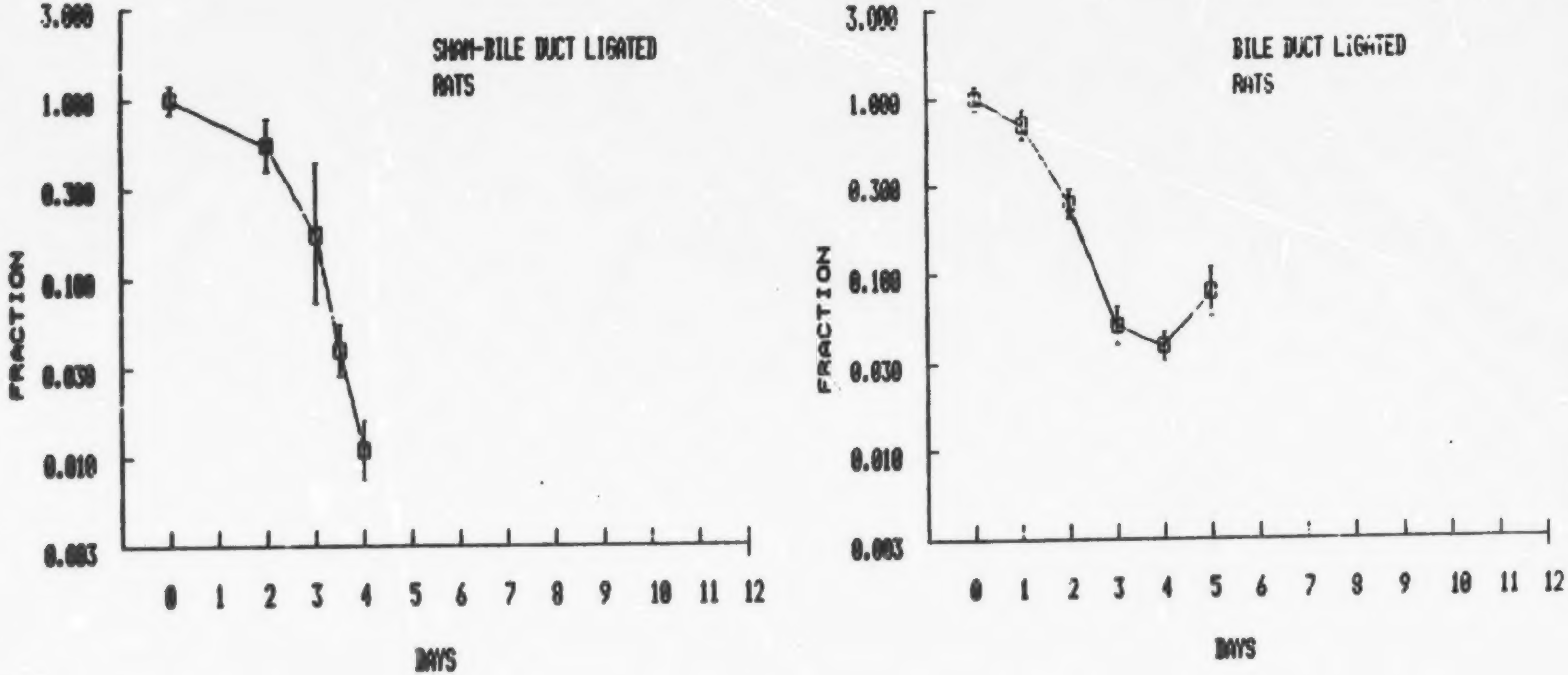

Figure 27. Plasma diamine oxidase (DAO) activity in 18 Gy, bile duct ligated and sham-bile duct ligated animals as a function of time after gamma irradiation. Each point represents the fraction of plasma DAO activity present in irradiated as compared to non-irradiated rats.

metabolic imbalances resulting from negligible food intake and excessive diarrhea after irradiation, (e) to evaluate the effectiveness of plasma transfusion to replace plasma protein and trace elements that leak from the intestine and are lost. In all these studies it is recommended that changes in plasma Na, K, and Zn, plasma DAO activity, plasma volume, blood cell counts (WBC, RBC, and platelet), hematocrit (HCT), liver function, kidney function, and lung function be monitored to assess the efficacy of these treatments in preventing intestinal radiation death with minimal damage to other organ systems. All fluids should be administered by continuous intravenous infusion rather than as a bolus.

A major advantage of using rats for such studies is that one can economically study a variety of fluid and electrolyte treatment schemes using a statistically significant number of animals. The most effective schemes could subsequently be tried on large animals. Finally, the most effective fluid and electrolyte treatment should ultimately be combined with established treatments to protect against the deleterious effects of hematopoietic injury produced by whole body irradiation. This includes platelet transfusions to combat hemorrhaging, systemic antibiotic and selective decontamination of the GI tract to combat infection, and bone marrow transplantation to regenerate the bone marrow. Efforts should also be made to look at the effectiveness of optimum fluid therapy combined with biological response modifiers, such as glucan, which can stimulate recovery of the hematopoietic system. The optimum fluid and electrolyte therapy should also be combined with treatment that stimulates recovery of the GI tract, such as administration of the regulatory peptides, glucagon and gastrin. These peptides have been shown to increase cell division in the intestinal mucosa (92).

In a nuclear weapon detonation a large fraction of the casualties will receive not only ionizing radiation exposures but also injuries from blast and thermal effects. These non-ionizing radiation traumas can result in sequestering large amounts of fluid and electrolytes in the injured areas of the body. This so called 'third space' results in a relatively nonfunctional extracellular fluid compartment that decreases the functional interstitial fluid and plasma volumes. As a consequence, radiation injury, when combined with other trauma, may result in death from hypovolemic shock at radiation doses that are significantly lower than those ordinarily required to produce intestinal radiation death. For example, rats exposed to a whole body dose of 12 Gy die from treatable hematopoietic injury, even though there is a drop in plasma volume 4 days after irradiation that is 90% of that seen in animals dying of intestinal radiation injury (Table 14). Consequently, a small but further decrease in plasma volume (e.g., another 10%) due to blast or thermal trauma could result in all animals dying from hypovolemic shock and shifting the radiation dose response curve by at least 6 Gy. In other words, burn or blast trauma may result in lethal hypovolemic shock at radiation doses that otherwise would result in the hematopoietic mode of death. This situation may also be aggravated by radiation damage delaying capillary refilling of the plasma compartment by fluid reabsorption from the blast or thermal zone of injury. Therefore, there is a need to study plasma volume changes as a function of time after irradiation in animals having combined injuries, such as radiation plus burn. Such information will be necessary to develop effective fluid therapy of these combined injuries.

SECTION 5

PUBLICATIONS AND PAPERS PRESENTED AT MEETINGS

The following publications and presentations are based on work conducted under this contract:

Geraci, J.P., Jackson, K.L., and Mariano, M.S., Absence of Sepsis and Endotoxin in Acute Intestinal Death Following Neutron Irradiation. (Abstract) Proceedings of the Seventh International Congress of Radiation Research, D4-11, July 3-8, Amsterdam, The Netherlands, 1983.

Geraci, J.P., Jackson, K.L., and Mariano, M.S., Effect of Antibiotic Decontamination of the GI Tract on Survival Time After Neutron and Gamma Irradiation. (Abstract) Health Physics 47:149 1984.

Jackson, K.L., Geraci, J.P., and Mariano, M.S., Effect of Bile Duct Ligation in Combination with Antibiotic Decontamination of the GI Tract on Survival Time after Neutron and Gamma Irradiation. (Abstract) Health Physics 47:150 1984.

Geraci, J.P., Jackson, K.L., and Mariano, M.S., Effects of Pseudomonas Contamination or Antibiotic Decontamination of the GI Tract on Acute Radiation Lethality. (Abstract) Radiation Research Society Meeting, Los Angeles, CA. May 4-9, 1985.

Geraci, J.P., Jackson, K.L., and Mariano, M.S., The Intestinal Radiation Syndrome: Sepsis and Endotoxin. Radiat. Res. 101:442-450 1985.

Geraci, J.P., Jackson, K.L., and Mariano, M.S., Effects of Pseudomonas Contamination or Antibiotic Decontamination of the GI Tract on Acute Radiation Lethality after Neutron or Gamma Irradiation. Radiat. Res. 104:395-405 1985.

Geraci, J.P., Dunston, S.G., Jackson, K.L., Mariano, M.S., Holeski, C., and Eaton, D.L., Bile Loss and Acute Intestinal Death in Rats. (Abstract) Radiation Research Society Meeting, Las Vegas, Nevada, April 13-17, 1986.

Geraci, J.P., Dunston, S.G., Jackson, K.L., Mariano, M.S., Holeski C., and Eaton D.L. Bile Loss in Acute Intestinal Radiation Syndrome in Rats. Submitted to Rad. Res.

Dunston S.G., Role of Bile in Radiation Induced Gastrointestinal Death in Rats (M.S. Thesis Radiological Sciences, J.P. Geraci preceptor) University of Washington, Seattle, WA 1984.

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APPENDIX A

WORK STATEMENT

Under this Contract, the Contractor, as an independent Contractor, and not as an agent, servant, or employee of the Government, utilizing special knowledge and techniques possessed by and available to the Contractor, shall furnish all labor, equipment, facilities, services, and materials, except as set forth under ARTICLE II, to undertake the following TASKS:

The overall objectives of this effort are to clarify the role of fluid and electrolyte loss, bile-duct ligation, radiation-damaged intestinal mucosa, bacterial toxemia and their interrelationships on radiation-induced gastrointestinal death. The relative effects of gamma rays and neutrons on these mechanisms will also be evaluated.

The research will be accomplished in a series of experiments outlined as follows:

Year 1 - Experiment 1: Preliminary studies to evaluate methods for antibiotic decontamination of GI tract.

Experiment 2: Damage to intestinal mucosa after gamma and neutron irradiation and correlation with levels of bacteria present.

Experiment 3: Effect of antibiotic decontamination on lethality and survival times after neutron and gamma irradiation.

Experiment 4: Effect of bile-duct ligation on survival time after neutron and gamma irradiation of normal and antibiotically-treated animals.

Year 2 - Experiment 5: Effect of specific bacteria known to cause diarrhea on sensitivity to acute intestinal radiation death.

Experiment 6: Changes in body fluid compartments in normal and antibiotically-treated animals after irradiation.

Year 3 - Experiment 8: Influence of bile-duct ligation on body fluid compartments after irradiation.

Experiment 9: Effect of bile-duct ligation on bacterial population in GI tract and endotoxin release after irradiation.

Experiment 10: Administration of antibiotics and saline into the GI tract and resultant effects on survival time after irradiation.

The details of the experimental protocols follow. Significant changes shall be accomplished by Change Orders or Supplemental Agreements.

Experiment 1: Year 1

Antibiotic decontamination of the alimentary tract. To insure that administration of antibiotics to rats decontaminate the alimentary tract, 30 rats will receive antibiotics in the drinking water for 2 weeks. At 4, 7, and 14 days fresh fecal samples will be analyzed microbiologically. It is estimated this preliminary study can be completed within 2 months.

Experiment 2:

Correlation of intestinal epithelium breakdown with sepsis or endotoxin after neutron or gamma irradiation. Conventional rats will be whole-body exposed to 500, 1,000, and 1,500 rad of gamma rays, or 180, 355, and 535 rad of neutrons. The neutron doses will be adjusted to produce approximately equal injury to the mucosa of the small intestine based on previous study with the 8 MeV neutron beam using DNA content of the small intestine 2 days after exposure as the endpoint. Each day for 7 days after irradiation, 10 neutron and 10 gamma irradiated rats at each dose will be sacrificed. At zero time 10 non-irradiated control animals also will be sacrificed. In all animals DNA content of the small intestine will be measured and the proportion of animals positive for endotoxin and/or sepsis in the blood and liver will be determined. From these data it will be determined if sepsis or endotoxin in the blood or liver correlates with radiation-induced breakdown of the mucosa of the small intestine following sublethal, lethal, and supralethal neutron and gamma-ray exposures. Those samples which are positive for sepsis will be sent to the Division of animal Medicine Diagnostic and Investigative Laboratory for identification of the organism(s) involved. This information will be important in determining if a single type organism is frequently involved in sepsis.

Endotoxin is expected to be present in the liver during the first few days after irradiation since mice x-irradiated with 850 rad have been reported to be positive for liver endotoxin 24 to 72 hours after exposure. A total of 430 rats will be used in this phase of the study which should be completed in 5 months.

Experiment 3:

LD 50 and survival time of conventional and decontaminated rats as a function of radiation dose of neutrons or gamma-rays.

This study is to determine the LD50 values for intestinal death and the time interval over which this should be measured. For this experiment 8 conventional and 8 decontaminated rats will be exposed to each of the following whole-body doses: 500, 750, 1,000 and 1,100, 1,200, 1,300, 1,400, 1,500, 1,600, 1,700, 1,800, 1,900, and 2,000 rad of gamma radiation and 200, 300, 400, 480, 520, 560, 600, 640, 680, 720, 760, and 800 rad of neutrons. The neutron doses have been adjusted to produce equal damage based on our previous measurement of the LD50/5 day for 8 MeV neutrons relative to x-rays, which gives an RBE of 2.4.

The animals will be followed to determine the time of death. If acute death does not occur, the animals will be followed for 30 days. During this period the animals will be checked at 8 hour intervals for the appearance of severe diarrhea, a clinical sign of impending intestinal death, and for the time of death. From these data curves will be constructed which show mean survival time and time of onset of diarrhea as a function of gamma-ray or neutron dose for conventional and decontaminated rats.

The data obtained in this phase of the study will allow direct calculation of the neutron RBE for intestinal death in decontaminated and conventional rats. A total of 416 rats will be used in this experiment which will require 3 months for completion.

Experiment 4:

Effect of bile duct ligation on survival time of supralethally neutron or gamma irradiated decontaminated and conventional rats.

Half of a group of rats will be bile duct ligated immediately after 1,500 rad whole-body gamma irradiation of equal numbers of conventional and decontaminated rats and after approximately 600 rad whole-body neutron irradiation of equal numbers of conventional and decontaminated rats. The above dose estimate for the neutron irradiation is based on an LD50/5 day RBE of 2.5. Data obtained in Experiment 3 above will be used to calculate the acute LD50 values for intestinal death at the appropriate times after irradiation of conventional and decontaminated rats in order to compute RBE values. RBE data from Experiment 3 will be used to adjust the neutron dose in the present experiment.

All rats will be checked at 8 hour intervals until death in order to record the times of onset of diarrhea and the times of death. From these data the effects of bile duct ligation on mean survival times of gamma and neutron supralethally irradiated rats will be obtained. This information will aid in determining if bile or bile acids interact with enteric organisms in the intestinal radiation syndrome produced by gamma or neutron irradiation. Previous work has shown that in conventional 1,500 R irradiated rats, bile duct ligation after irradiation increases mean survival time from 3.3 days.

Experiment 5: Year 2

Can a specific enteric microorganism, (to be determined by University of Washington and AFRRI investigators), which is known to cause diarrhea and is probably present in some humans who exhibit chronic, intermittent diarrhea, increase sensitivity to acute intestinal radiation death? One hundred conventional rats will be divided into two groups. One group of 20 rats will be orally intubated with 2 ml of sterile saline. The remaining animals, in groups of 20, will be orally intubated with 2 ml of saline containing the selected microorganism. The number of organisms administered will be increased progressively in the 4 groups receiving the bacteria up to the maximum number that have no effect in producing diarrhea in nonirradiated rats. One day after intubation all animals will be wholebody gamma irradiated at the previously measured LD 5/5 day for conventional rats. (The dose required to produce five percent mortality will be calculated from data obtained in the first experiment.) Particular attention will be paid to the time of onset of diarrhea among animals of the various groups.

Subsequent to completion of this pilot experiment, rats will be intubated with the minimum number of organisms that increases mortality to 100% in conventional rats receiving an LD5/5 day radiation dose. Then the radiation LD50/5 day of these intubated rats will be measured and compared with the radiation LD50/5 day of conventional rats. The difference will be interpreted as a quantitative measure of the change in radiosensitivity to acute intestinal death caused by the selected organism. Approximately 80 irradiated rats will be used for this second phase of the experiment.

Experiment 6:

Changes in body fluid compartment volumes in conventional and decontaminated rats after sublethal, lethal and supralethal irradiation. Equal numbers of conventional and decontaminated rats will receive whole body gamma radiation doses of 500, 1,000, and 1,500 rad. (These doses may be altered somewhat depending on the results obtained in Experiment 3 which will give data on survival time as a function of radiation dose for conventional and decontaminated rats.) The radiation doses selected will be those which are sublethal, 100% lethal at 30 days and doses somewhat greater than the thresholds for intestinal death. On the day before irradiation and on 1, 2, 3, 4, 5. and 6 days after irradiation (or to the time of death) 5 conventional rats and 5 decontaminated rats will be subjected to measurement of total body water, extra cellular fluid space (inulin space), red cell volume, and plasma volume. This information will indicate whether major shifts in fluid and electrolyte occur in the irradiated animal, whether these are related to intestinal death, and whether the enteric microflora plays a role in fluid shifts.

For this phase of the study isotope dilution measurements will be used to determine fluid compartment sizes. Increased amounts of fluid can be in the lumen of the GI tract after supralethal irradiation and there may be incomplete equilibration of isotopes with the fluid contained in the gut when measurements are made of extracellular fluid space and total body water. Also there can be leakage of large labeled molecules into the lumen of the GI tract after irradiation. Consequently, in each isotope dilution experiment, after obtaining blood samples, the total amount of tracer in the GI tract will be measured separately by removing the GI tract and determining its total radioactivity content. This will allow estimates to be made of the size of body fluid compartments, excluding the GI tract. This approach is to obtain physiologically pertinent estimates of radiation-induced changes in fluid compartments that may be responsible for shock and death.

Measurement of circulating red cell volume and total plasma volume will be conducted according to the methods recommended for measurement of these parameters by the International Committee for Standardization in Hematology, with modification for use in rats. Total blood volume will be taken as the sum of these two values. Rat erythrocytes will be labeled with 50-Cr as sodium chromate using the citrate wash method. Commercially available 125-I human albumin will be used to measure plasma volume. With the rat under ether anesthesia, a known amount of activity of each of these tracers will be intravenously injected via the external jugular vein. Three minutes after injection blood will be withdrawn from the exposed heat, followed by removal of the GI tract for analysis. 51-Cr and 125-I will be measured with a sodium iodide crystal, gamma spectrometer.

An estimate of the extracellular fluid space (inulin space) will be obtained with 14-C-insulin using the method of Zsebok and Petranyi. Rats will be surgically opened under ether anesthesia and two renal hila ligated. The abdomen will be closed with surgical clips and the 14-C-insulin injected through the jugular vein. After 30 minutes, a sample of cardiac blood will be taken for 14-C analysis of the plasma by liquid scintillation spectrometry. Appropriate quenching corrections will be made by recounting samples after adding an internal standard of 14-C-insulin.

Total body water will be based on methods developed for rats. Rats will receive an intravenous injection of tritiated water as isotonic saline solution via the jugular vein. After 1 hour a cardiac blood sample will be taken and the plasma analyzed for tritium content by liquid scintillation counting. Tritiated water loss in the urine during a 1 hour period is negligible with respect to the value obtained for total body water. During liquid scintillation analysis corrections for quenching will be made by recounting samples after addition of an internal tritium standard. For the body fluid compartment measurements it will be necessary to repeat the experiments three times in order to measure 1) red cell and plasma volumes, 2) inulin space, and 3) total body water. For this purpose a total of 540 rats will be used. This experiment will require 9 months for completion.

Experiment 8: Year 3

Influence of bile duct ligation on body fluid compartment changes after supralethal irradiation. In this experiment only those fluid compartment which show major changes after 1,500 rad gamma irradiation (determined in Experiment 6) will be investigated. Half of a group of conventional rats will be bile duct ligated at the time there is a maximum effect on survival time. All rats will be exposed to 1,500 rad of gamma-radiation. On the day before irradiation and on days 1, 2, 3, 4, 5 and 6 (or to the time of death) 5 conventional rats and 5 bile duct ligated rats will be subjected to body fluid compartment measurements. Approximately 140 rats will be required for this experiment which will require 5 months for completion. These data will contribute to an understanding of the importance of fluid and electrolyte changes in the mechanism of intestinal radiation death, and therefore are of importance in the selection of appropriate medical treatment.

Experiment 9: **(Note that this experiment was deleted and a substitute Experiment 9 was added to the Work Statement as described subsequently.)**

Influence of bile duct ligation on sepsis and endotoxin release following supralethal irradiation. If sepsis and/or endotoxin release from the intestine is the primary cause of intestinal radiation death rather than fluid and electrolyte loss, bile duct ligation (which increases survival time) should alter the time of onset and/or severity of endotoxin release or sepsis. To test for this, half of a group of conventional rats will be bile duct ligated one day before irradiation. All rats will be whole-body exposed to 1,500 rad of gamma radiation. On days 0, 1, 2, 3, 4, in the non-bile duct ligated group and on days 0, 1, 2, 3, 4, 5 and 6 in the bile duct ligated group, samples of blood and liver will be cultured to determine if and when sepsis occurs. The livers and blood also will be assayed for endotoxin. A total of 96 rats will be used in this experiment which will require 4 months for completion. This information will help to clarify the relative importance of enteric bacterial organisms as compared to fluid and electrolyte loss in the mechanism of acute intestinal radiation death.

Experiment 10:

Effect on survival time of antibiotics and saline delivered to the GI tract post-irradiation. The purpose of this experiment is a practical one, to determine if intubation of antibiotics plus saline directly into the small intestine after 1,000 or 1,500 rad whole-body irradiation can significantly increase survival time.

Conventional rats will be whole-body exposed to 1,000 or 1,500 rad of gamma radiation. Immediately after irradiation each animal will receive one of four types of injection until the time of death: (a) isotonic glucose solution, (b) isotonic glucose solution containing antibiotics at the concentrations (first week) used previously in the drinking water for oral decontamination of the GI tract, (c) isotonic saline, or (d) isotonic saline containing antibiotics at the concentrations (first week) used in the drinking water for oral decontamination of the GI tract.

To administer these solutions directly into the intestine, immediately before irradiation a small silastic tubing cannula will be sutured into the upper duodenum, running under the skin and exiting behind the neck. Each day, commencing immediately after irradiation, a volume of fluid (a), (b), (c), or (d), equal to the normal daily water intake, will be administered via the cannula in two daily increments. The animals will be checked at 8 hour intervals to determine times of death. The endpoint will be change in mean survival time relative to the glucose injected irradiated control (a). These data will indicate whether antibiotics plus saline injection into the intestine can increase survival time of lethally and supralethally irradiated rats and whether there is an interaction between electrolyte administration and antibiotic administration. This experiment will require 100 rats and will be completed in 3 months.

AFRRI Experiment - The effectiveness of antibiotic decontamination of the GI tract and bile duct ligation for extending survival time and increasing LD50 after exposure to neutrons and gamma rays from the AFRRI TRIGA reactor will be evaluated.

Experimental Protocols: The experiments to be conducted will include the following groups of rats:

1. Conventional - controls
2. Decontaminated
3. Conventional - bile duct ligated
4. Decontaminated - bile duct ligated
5. Conventional - sham bile duct ligated
6. Decontaminated - sham bile duct ligated

The same rat species, sex and sized animals will be used as previously used at the University of Washington. All procedures will be controlled as closely as possible to allow for as accurate an assessment as possible of the relative effects of the different energy neutrons from the University of Washington Cyclotron with the AFRRI TRIGA reactor on the mechanisms being studied; i.e., influence of bile salts and enteric organisms on survival time and LD50.

For each exposure group, LD50 values for hematopoietic and intestinal death and mean and median survival times and times of onset of diarrhea as a function of neutron dose will be assessed. The median survival time of decontaminated and conventional rats following gamma irradiation at a single dose as control for comparison with the data obtained at the University of Washington will be obtained.

At the end of the experiments, the data obtained will be described and provided in the next quarterly report due after return to the University of Washington. An analysis of these data with data obtained from previous work at the University of Washington will be performed and a report provided with the 2nd quarterly report due after the end of these experiments.

b. Under ITEM I - Work Statement delete Experiment 9 and substitute the following:

Experiment 9 - Role of Bile Acids in Acute Intestinal Radiation Death.

Experiment Protocols:

Introduction:

Ligation of the bile duct of rats significantly prolongs survival in animals destined to die from acute intestinal radiation injury. Two mechanisms have been proposed for this phenomenon. One hypothesis is that ligation slows down the loss of fluid and electrolytes by stopping the flow of these materials into the intestine via the bile, therefore prolonging survival by reducing fluid and electrolyte loss from the body in the form of diarrhea. A second possible mechanism is the bile duct ligation prevents toxic bile acids from reaching the intestine, which may reduce intestinal damage. The purpose of this experiment is to obtain information which will help determine which of these hypotheses is correct. This will be accomplished by comparing survival times [illegible] irradiated, bile duct ligated rats and sham bile duct ligated rats which have had either bile acid or a control solution injected directly into the intestine.

Procedure:

Cannula, originating at the back of the neck, will be inserted into the upper duodenum of 90 rats one week prior to irradiation. All cannulated rats will be exposed to 16 Gy of whole-body gamma radiation. Following irradiation the animals will be divided into 6 treatment groups of 15 animals each. These six groups are listed below (BDL = bile duct ligated, SBDL = sham bile duct ligated):

1. BDL rats receiving lactate-Ringer solution containing 0.38 M taurocholic acid

2. BDL rats receiving lactate-Ringer solution containing 0.19 M taurocholic acid

3. BDL rats receiving lactate-Ringer solution

4. SBDL rats receiving lactate-Ringer solution containing 0.38 M taurocholic acid

5. SBDL rats receiving lactate-Ringer solution containing 0.19 M taurocholic acid

6. SBDL rats receiving lactate-Ringer solution

At 8 hour intervals 3 ml of taurocholic acid in lactate-Ringer solution or lactate-Ringer solution will be injected into the upper small intestine via the cannula. Following irradiation the animals will be checked at 4 hour intervals to determine the time of onset of diarrhea and lethality. Analysis of variance will be used to determine if there is a statistically significant change in the median survival time among the treatment groups. From these data it should be possible to determine if bile acids enhance and/or accelerate development of radiation injury in the intestinal tract.

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APPENDIX B

University of Washington Correspondence

INTERDEPARTMENTAL

Medical Radiation Physics
NN136 University Hospital, RC-08

January 4, 1984

TO: Ken Jackson
Joe Geraci
Radiological Sciences, SB-75

FROM: Juri Eenmaa Jüri
Medical Radiation Physics, RC-08

SUBJECT: Radiobiology Dosimetry

See attached report.

JE/ba
Enclosure
[CNT]M-363

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Report on Measurements of Neutron/Gamma Dose Fractions using TE(air) and Mg(Ar) Ion Chambers.

This report presents the results of determinations of neutron/gamma dose fractions for radiobiology using paired chambers.

The following information is included:

- Instrumentation
- Neutron Beam Measurements
- Neutron Beam Dose
- Calculations
- Final Results
- Appendix (Calculation of $\bar{k}_U$)

Instrumentation

(1) Tissue-equivalent chamber:

Far West Technology
Model IC-17, A-150 Wall
Air chamber gas
Co-60 equilibrium cap.
1cc volume
N_C (Co-60) $= 3.291 \times 10^9$ R/coul
$= 3.126 \times 10^9$ rad/coul
N_C (Cs-137) $= 3.314 \times 10^9$ R/coul

(2) Magnesium chamber:

Exradin
MG-2, Magnesium wall
Argon chamber gas (5cc/min flow)
0.5cc volume
3mm equilibrium cap
Bias volts: + 300V
N_C(Cs-137) $= 3.690 \times 10^9$ R/coul
$= 3.70 \times 10^9$ rad/coul
N_C(Co-60) $= 3.67 \times 10^9$ rad/coul (by inference)

(3) Electrometer:

Princeton Applied Research
Model 135; Serial No. 172
$C_E = 1.009 \times 10^{-8}$ (Coulomb scale)

Neutron Beam Measurements

See Figure 1 for set-up schematic

TE Chamber

Position	V/TGT BIC	C_E	K_{TP}	Q/TGT BIC
1	.0302	1.009×10^{-8}	1.006	3.076×10^{-10}
2	.0334	"	"	3.390
3	.0344	"	"	3.492
4	.0329	"	"	3.340
Ave	-	-	-	3.323×10^{-10}

Magnesium Chamber

Position	V/TGT BIC	C_E	K_{TP}	Q/TGT BIC
1	.00357	1.009×10^{-8}	1.006	0.362×10^{-10}
2	.00402	"	"	0.408
3	.00420	"	"	0.426
4	.00395	"	"	0.401
Ave	-	-	-	0.399×10^{-10}

Neutron Beam Measurements (Summary)

Position	TE/AIR (coul/TGT BIC	MG/AR (coul/TGT BIC
1	3.070×10^{-10}	0.362×10^{-10}
2	3.390	0.408
3	3.492	0.426
4	3.340	0.401
Ave	3.323×10^{-10}	0.399×10^{-10}

Neutron Beam Dose

The dose components at a point in the mixed field can be computed from the response of the two dosimeters by the use of the following simultaneous equations:

$$R_T^1 = k_T D_N + h_T D_G$$

$$R_U^1 = k_U D_N + h_U D_G$$

The separate neutron absorbed dose, D_N, and the gamma-ray absorbed dose, D_G, are then given by:

$$D_N = \frac{h_U R_T^1 - h_T R_U^1}{h_U k_T - h_T k_U}$$

$$D_G = \frac{k_T R_U^1 - k_U R_T^1}{h_U k_T - h_T k_U}$$

In these equations, the subscript T refers to the TE chamber and the subscript U refers to the neutron-insensitive (Magnesium) chamber. R_T^1 and R_U^1 are the quotients of the responses of the two dosimeters in the same mixed beam relative to their sensitivities (the ratio of the dosimeter response to dose measured) to the gamma rays used for the photon calibration. Similarly, k_T and k_U are ratios of the sensitivities of each dosimeter to neutrons relative to their sensitivity to the gamma rays used in calibration, and h_T and h_U are the ratios of the sensitivities of each dosimeter to the photons in the mixed field relative to their sensitivity to the gamma rays used for calibration. The values of h_T and h_U are close to unity and the assumption $h_T = h_U = 1$ is usually made. The values of k_T and k_U for the UW neutron beam and for the two chambers, are given by:

$$k_T = \frac{C^W}{W_N} \frac{C^S}{S_N} \frac{C^K}{k_N} \times \frac{1}{k_d}$$

$$= \frac{33.7}{35.8} \times \frac{1.133}{1.188} \times \frac{1.004}{.954} \times \frac{1}{0.97}$$

$$= 0.974$$

$$k_U = \bar{k}_U / k_d$$

$$= 0.106/k_d$$

$$= 0.106/0.987$$

$$= 0.107$$

Note: See appendix for calculations of $\bar{k}_U$.

Note: The recommended value of the displacement correction factor, for measurements in phantom, is included in the above for the two chambers.

Calculations

$h_U = 1$

$h_T = 1$

$k_T = 0.974$

$k_U = 0.107$

$R_T^1 = 3.323 \times 10^{-10}$ coul/TGT BIC $\times\ 3.126 \times 10^{9}$ rad/coul

$= 1.039$ rad/TGT BIC

$R_U^1 = 0.399 \times 10^{-10}$ coul/TGT BIC $\times\ 3.67 \times 10^{9}$ rad/coul

$= 0.146$ rad/TGT BIC

$$D_N = \frac{(1)(1.039)-(1)(0.146)}{(1)(0.974)-(1)(0.107)}$$

$= 1.030$ rad/TGT BIC

$$D_G = \frac{(1)(0.146)-(0.107)(1.039)}{(1)(0.974)-(1)(0.107)}$$

$= 0.040$

Final Results

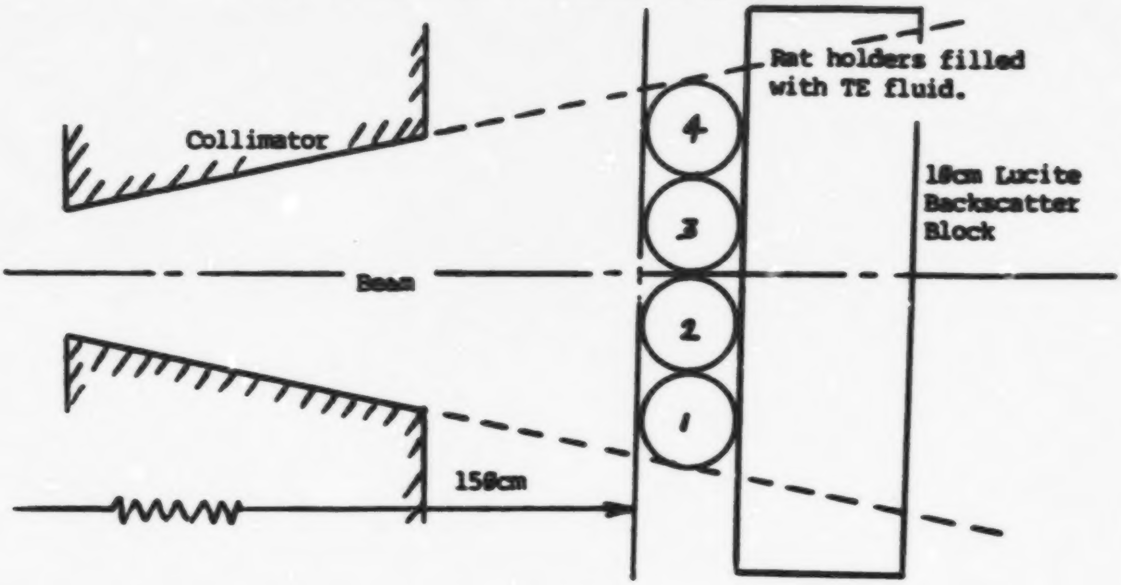
$D_{TOTAL} = 1.070$ Rad/TGT BIC

$D_N = 1.03$ Rad/TGT BIC

$D_G = 0.04$ Rad/TGT BIC
(approx 4% of the total dose)

Note: This result is consistent in the measurements made with a TE proportional counter (Med Phys 4, 379-386, 1977) on the same neutron beam. The TE proportional counter gave a result of about 7% at 2-cm depth in a 30-cm cubical phantom for a 22 x 22 cm x cm field size. In this latter case one would expect more backscatter from the solid phantom, than from the rat phantom.

Figure 1. Neutron Beam Measurements Set-up.


Rat holders filled with TE fluid.
Collimator
4
3
10cm Lucite Backscatter Block
Beam
2
1
150cm

Field size: 23.5 x 23.5 cm x cm at 150cm

Chamber position: At center of rat holders.

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Appendix: Calculation of k_U

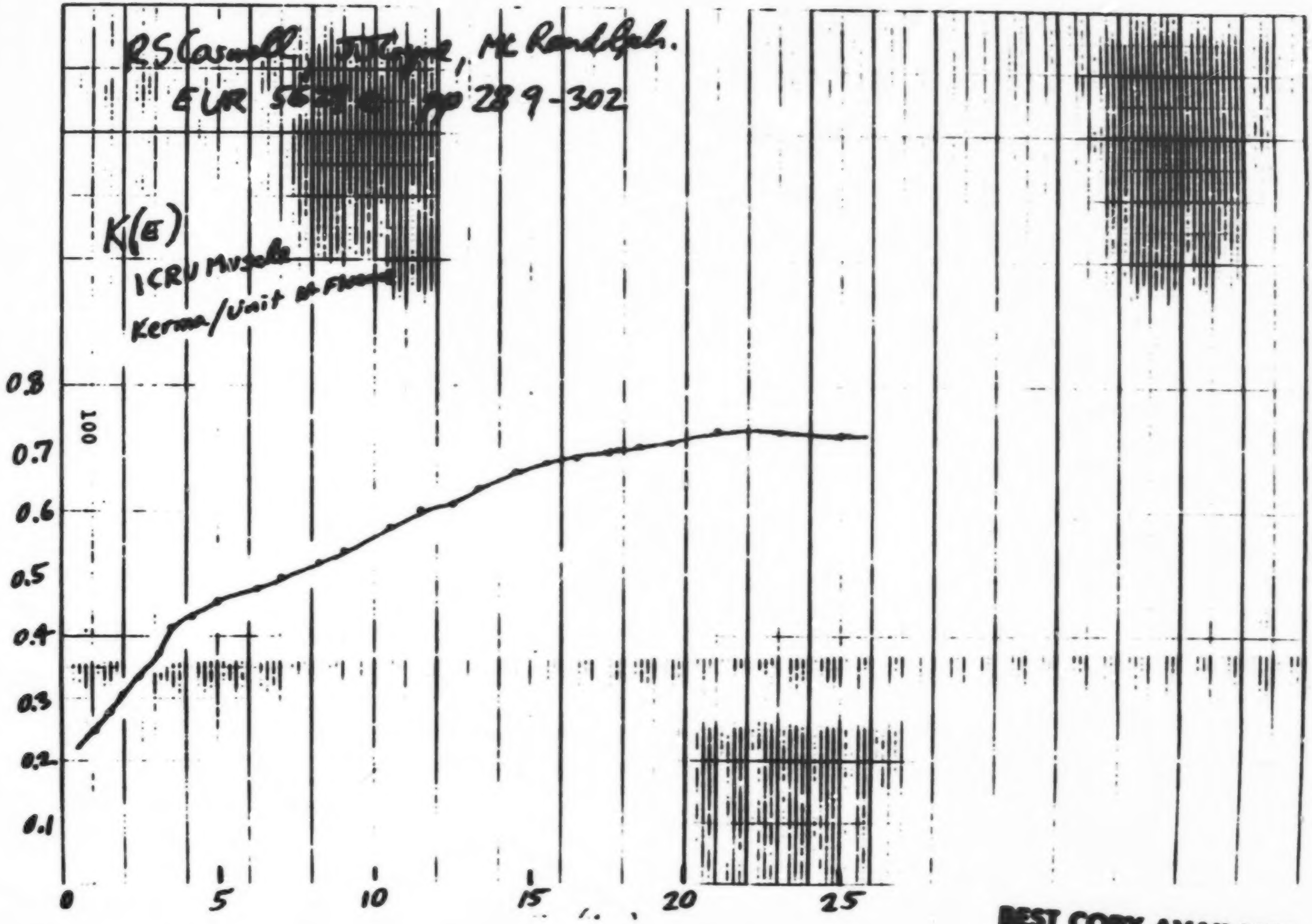
$$\overline{k}_U = \frac{\int k_U(E)\, \phi(E)\, K(E)\, dE}{\int \phi(E)\, K(E)\, dE}$$

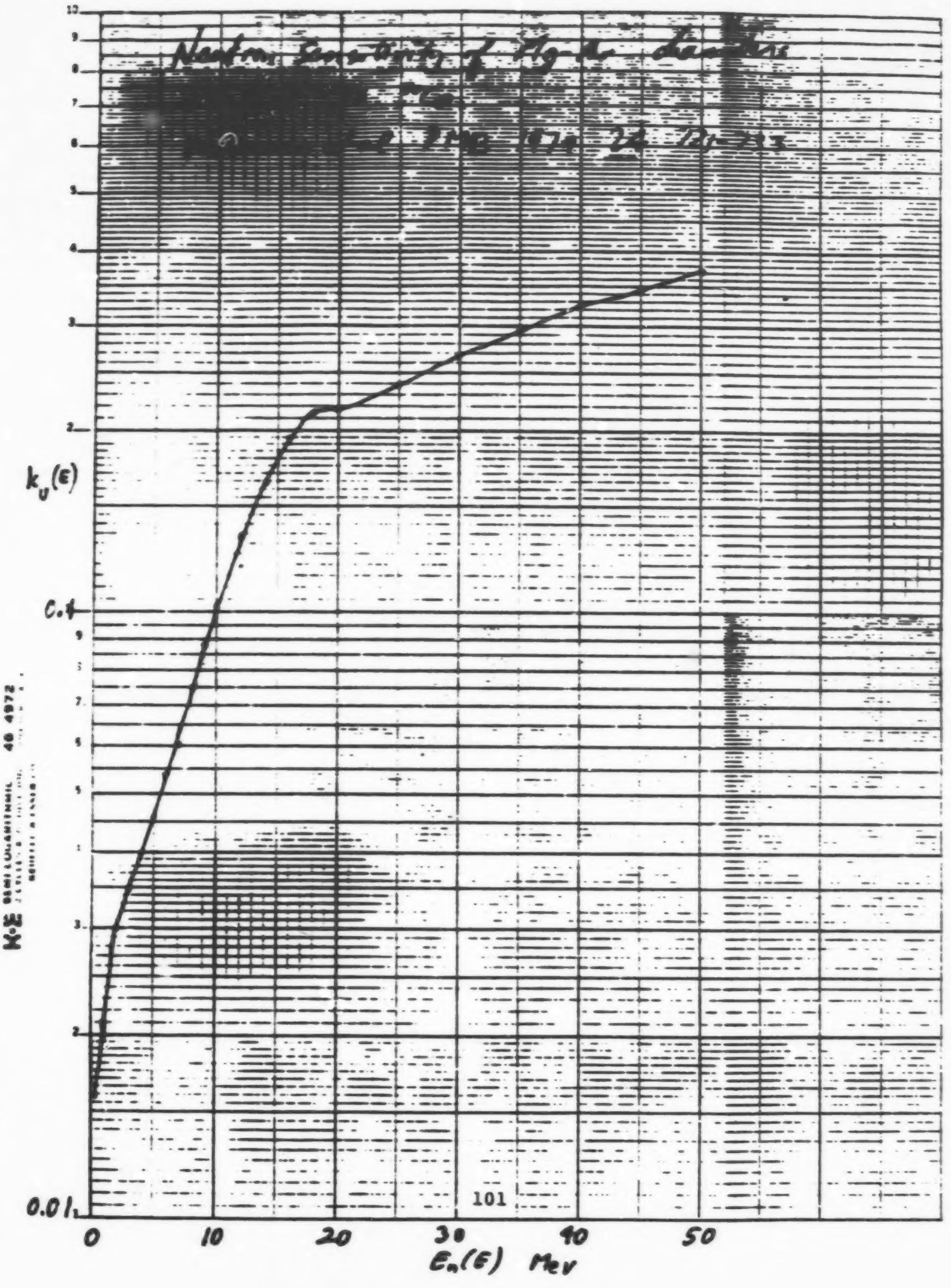
where $k_U(E)$ = relative neutron sensitivity of the neutron insensitive chamber (Mg-Ar), as a function of neutron energy (Waterman et al., 1979)*

$\phi(E)$ = neutron differential spectrum of fluence.

$K(E)$ = tissue kerma factor

* Note: relative to ^{60}Co gamma rays.
Estimate uncertainty to be ±10% below 20Me.
and ±15% above 35 MeV.
Data are for
0.5 cm^3 thimble Mg chamber
with continuously flowing Argon gas
Used 4 mm thick Mg-buildup cap to
provide a total wall thickness of 5 mm.
±300 V bias.


R S Caswell, J J Coyne, M C Randolph.
EUR 56[illegible] pp 289-302
K(E)
ICRU Muscle
Kerma/Unit of Fluence
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
5
10
15
20
25


Neutron Sensitivity of Hg-U chambers
1974 24 121-323
$k_U(E)$
0.01
0.1
0
10
20
30
40
50
$E_n(E)$ MeV
K-E SEMI-LOGARITHMIC 46 4972

E_n	ΔE_n	$\phi(E)$	$K(E)$	$k_u(E)$
—	—	—	—	—
1	1 (.5-1.5)	.0065	.250	.021
2	1 (1.5-2.5)	.0139	.305	.030
3	1 (etc.)	.0223	.365	.035
4	1	.0309	.430	.040
5	1	.0387	.455	.046
6	1	.0454	.470	.054
7	1	.0482	.495	.060
8	1	.0483	.515	.075
9	1 (8.5-9.5)	.0463	.535	.088
10	1.5 (9.5-11)	.0410	.565	.103
12	2 (11-13)	.0320	.600	.134
14	2 (13-15)	.0210	.650	.165
16	2 (15-17)	.0126	.680	.195
18	2 (17-19)	.0070	.700	.215
20	2 (19-21)	.0040	.715	.218
22	2 (21-23)	.0020	.725	.228
24	2 (23-25)	.0010	.725	.233
26				

↑ d(21)Be differential energy spectrum (T o K).

↑ Tissue Kerma per unit fluence

↑ Waterman et al.

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	$\int \phi(E)\, K(E)\, dE$	$\int \phi(\epsilon)\, K(\epsilon)\, k_u(\epsilon)\, d\epsilon$
1	.00163	.00003
2	.00424	.00013
3	.00814	.00028
4	.01329	.00053
5	.01761	.00081
6	.02134	.00115
7	.02386	.00143
8	.02487	.00187
9	.02477	.00218
10	.03475	.00358
12	.03840	.00515
14	.02730	.00450
16	.01714	.00334
18	.00980	.00211
20	.00572	.00125
22	.00290	.00065
24	.00145	.00034
	.27721	.02933

$$\overline{k_U} = \frac{\int \phi(E)\, K(E)\, k_U(E)\, dE}{\int \phi(E)\, K(E)\, dE} = \frac{.02933}{.27721} = 0.1058$$

$\pm 10\%$

Nov 1, 1983

Cs-137 calibration for Ken Jackson and Joe Geraci UW.

Chamber: 188TG (air gas) 1cc A-150 wall
Electrometer: PAR 135 #172 (10^{-8}) $C_E = 1.009 \times 10^{-8}$

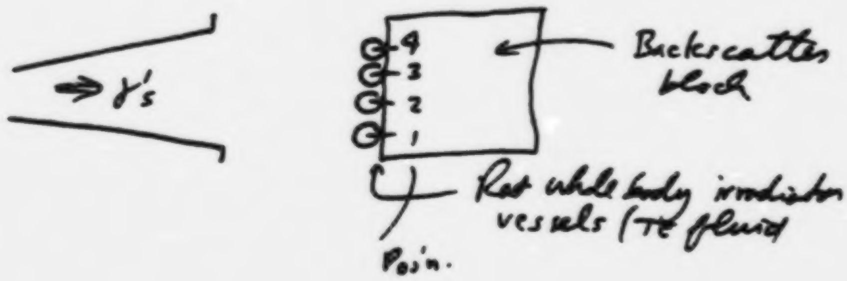
Chamber calibration: ADCL Wisconsin Sept. 1983
$N_C(\text{air}) = 3.291 \times 10^9$ R/coul

System Calibration

$$3.291 \times 10^9 \text{ R/coul} \times 1.009 \times 10^{-8} \text{ coul/volt} \times 0.95 \left(\text{rad/R}\right)_{\substack{\text{Cs-137}\\ \text{water}}}$$

$$= 3.155 \times 10^1 = \boxed{31.5_5 \text{ rad/volt}}$$

Setup:


γ's
4
3
2
1
Backscatter block
Rat whole body irradiator vessels (TE fluid
Posn.

T = 21.0 in fluid
P = 755.0
$K_{TP} = 1.003$

Chamber leakage: < 0.3 mV/min

Pos'n	Timer Set	Exposure (Volts)		Ave Exposure × K_{TP} (Volts)
1	2.00	2.445 2.446	2.445_5	2.453 / 2 min
2	2.00	2.663 2.664	2.663_5	2.671 / 2 min
3	2.00	2.653 2.652	2.652_5	2.660 / 2 min
4	2.00	2.475 4.476	2.755_5	2.483 / 2 min

Shutter Correction

t = timer setting

ΔT = shutter correction

$T = t + \Delta T$ true exposure time

x = exposure rate

X = observed exposure in time t $= x(t+\Delta T)$

4 short exposures (cumulative reading)

$t = 1$	X	
1.00	1.2352	
1.00	2.464	
1.00	3.693	
1.00	4.923	$= E_2$

1 long exposure

$t = 4$	X	
4.00	4.970	$= E_1$

$$\Delta T = \frac{4 \cdot 1 \cdot (E_2 - E_1)}{4E_1 - E_2} = \frac{-0.188}{14.957}$$

$$\boxed{\Delta T = -0.013}$$

shutter closes early!

So, for a timer setting of 2.00 minutes, the true exposure [redacted] time is: 2.00 - 0.013 = 1.987 min.

Summary of Results

Position	Dose Rate (rad/min)
1	31.55 × 2.453 / 1.987 = 38.95
2	31.55 × 2.671 / 1.987 = 42.41
3	31.55 × 2.660 / 1.987 = 42.24
4	31.55 × 2.483 / 1.987 = 39.43
	mean = 40.75

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